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A Safety Profile Analysis of Siddha Polyherbal Formulation *Ēlāti Cūraṇam*

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Abstract

The present study was conducted to evaluate the safety profile of the Siddha formulation *Ēlāti Cūraṇam* (EC) for managing hypertension by assessing its acute and subacute toxicity in Wistar albino rats. Prior approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC No: 04/321/PO/Re/S/01/CPCSEA-12/OCT-2018). For acute toxicity assessment, EC was administered as a single high dose of 2000 mg/kg, and the animals were observed for any immediate adverse reactions or signs of toxicity. For subacute toxicity evaluation, EC was given orally at doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg over a prolonged period. Haematological and biochemical analyses were performed to monitor potential changes in organ function and blood parameters. The results revealed that EC was non-toxic at the acute dose of 2000 mg/kg, with no notable adverse effects observed on vital organs. Similarly, repeated administration at subacute doses did not cause significant alterations in haematological or biochemical parameters. No severe toxicological effects were detected in the organs or physiological indices assessed.

Major Findings: The study found that *Ēlāti Cūraṇam* was non-toxic at a high acute dose of 2000 mg/kg and showed no significant adverse effects at subacute doses (100, 150, 200 mg/kg). Haematological, biochemical, and organ assessments confirmed its safety profile, supporting its traditional use for long-term management of hypertension. In conclusion, the findings suggest that EC is safe under both acute and subacute exposure conditions. These toxicological outcomes provide supportive evidence for the long-term use of EC and validate its traditional application from a safety standpoint.

Keywords: Acute Toxicity, *Ēlāti Cūraṇam*, Siddha, Sub-Acute Toxicity

1. Introduction

The field of toxicology involves studying how chemicals, substances, or conditions can negatively impact living organisms and the environment. Often referred to as the "Science of Safety", toxicology has evolved from studying poisons and adverse chemical exposures to a broader focus on ensuring safety[1]. The specialty area of pharmacology known as "safety pharmacology" is devoted to determining and describing a substance's pharmacological properties that may affect its clinical safety. Evaluation of the drug's effects on the respiratory, central nervous, and cardiovascular systems—collectively referred to as the core battery of safety

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pharmacology tests—is crucial, according to established recommendations[2]. Understanding toxicology in Siddha medicine is crucial for ensuring the safety and efficacy of its treatments. Along with evaluating the toxicity of medicinal plants and their active chemicals, it is essential to consider the traditional usage of plant-based medicines in both people and animals to guarantee their safe use[3].

Siddha medicine is a classical system from South India that highlights the balance of humour known as *"Uyir tātukaḷ"* and the concept of *"Siddha Maruthuvam"* for treating various ailments. Despite having centuries of traditional usage, siddha medicines—including polyherbal formulations—need thorough toxicity studies to guarantee their safety, effectiveness, and widespread acceptability. When it comes to complicated multi-herbal and mineral compositions that might result in unanticipated interactions, include heavy metals, or vary in potency owing to uneven raw material quality, traditional usage alone does not ensure safety. Toxicity studies aid in determining acceptable dose levels, identifying possible side effects, and confirming the effectiveness of metal or mineral purification techniques.

Additionally, they are necessary for scientific legitimacy, public health protection, and regulatory approval, which together strengthen faith in Siddha treatment by bringing its age-old knowledge into line with contemporary safety regulations. Combining natural remedies can offer numerous therapeutic advantages due to their varied pharmacological effects and minimal toxicity, particularly when thoroughly assessed for safety. The term "*Cūraṇam*" describes a powdered medication composed of one or more medicinal plant components. It is used in Siddha practice and is well-known for being inexpensive and easy to use[4]. It is a traditional treatment that is described in the book *Sarabenthirar Vaithya Muraikal*[5]. This study focuses on the toxicological assessment of EC, a Siddha polyherbal compound used to treat hypertension.

2. Materials and Methods

2.1 Interventional Drug *Ēlāti Cūraṇam*

Elettaria cardamomum seeds, dried *Nymphaea nouchali* and *Nelumbo nucifera* flowers, *Cyperus rotundus* rhizome, *Glycyrrhiza glabra* root, *Syzygium aromaticum* flower buds, *Ziziphus mauritiana* seeds, *Dryobalanops aromatica* seeds, and fried *Oryza sativa* paddy are used to make EC. Each component is cleansed separately, dried in the shade to eliminate moisture, roasted, ground coarsely, and sieved as per Siddha literature. The final formulation is then created by carefully mixing the processed ingredients. The recommended dosage of EC is 1 *kazhanju* (5.1 g), to be administered with honey. It is indicated for the management of *Iratta Pittam* (Hypertension).

2.2 Preparation of Drug Solution

First, 1 g of EC powder was weighed using a precision balance. Then, a sanitised beaker or mixing container was filled with 10 millilitres of distilled water. The EC powder was slowly added to the distilled water and stirred until dissolved. A magnetic stirrer was used for thorough mixing when needed. Next, 10 ml of the 2% Carboxymethyl Cellulose (CMC) solution was measured and slowly added to the EC and distilled water mixture. It was thoroughly stirred to ensure the even distribution of the CMC and complete mixing with the EC. The mixture was agitated until homogeneous, and the EC was well dispersed in the CMC solution. CMC acted as a thickening agent and emulsifier to ensure the even dispersion of EC and prevent it from settling out of the solution.

2.2.1 Animals

To determine the potency of a drug, three adult Wistar female albino rats weighing between 150 to 200 grams each were employed for an acute-level toxicity study. Forty-eight albino rats (24 males and 24 females) were obtained from TANUVAS, Madhavaram, Chennai, to evaluate the substance's repeated oral strength. All animals were housed in regular circumstances (22±3°C), fed conventional pellet feed, and had unrestricted access to water. The Institutional Animal Ethics Committee approved, and the animal studies were conducted as per the Committee for Control and Supervision of Studies on Animals (CPCSEA) guidelines. With IAEC Number 04/321/PO/Re/S/01/CPCSEA-12/OCT-2018, the CPCSEA Institutional Animal Ethical Committee (IAEC) approved the experimental technique.

2.3 Preparation of Experimental Animals

Wistar albino rats that were young and healthy were selected for the investigation. The female rats had never given birth and were not pregnant. Each rat weighed between 150 and 250 grams at the start of the trial, with a maximum weight difference of 20% from the average weight of animals that

had previously received doses. The rats ranged in age from six to eight weeks. At 22°C±3°C, the rats were kept in a room with a relative humidity range of 30% to 70%, ideally between 50 and 60%, unless the space was being cleaned. Twelve hours of light and twelve hours of darkness were given to the rats. They had unrestricted access to water and were fed conventional laboratory meals. To guarantee enough rats for clear observation, the rats were divided into enclosures according to their dose. Before treatment, the rats were acclimated in their cages for at least seven days, randomly selected, and individually tagged for identification. The rats were given water for 16-18 h but no food for the whole night before being given EC.

2.4 Acute-Oral Toxicity Study

In compliance with OECD standards 423[6], three sexually mature Wistar female albino rats were given a single oral dose of 2000 mg/kg b.w. of EC, suspended in 2% CMC. The rats were fasted overnight but were allowed access to water for 16-18 h before treatment. The test drug EC was administered via oral gavage using a feeding needle. An equal portion of the vehicle was given to the vehicle control group. The rats were not fed for three to four hours following medication.

Skin discolouration, mobility, hostility, sensitivity to pain and sound, and respiratory movements were among the toxicity-related observations that were methodically documented. After 24 hours, the number of survivors was recorded, and for the following fourteen days, the rats were monitored each day for food consumption, body mass, and water intake.

2.5 Sub-Acute Oral Toxicity

OECD standards 407 [7] were followed in this investigation. There were twelve rats in each group—six males and six females. While the other three groups received dosages of 100, 150 and 200 mg/kg body weight of EC, respectively, the first group was given 2 ml/kg of the vehicle as a control. For 28 days, the test suspensions were prepared fresh daily and administered orally once a day by gavage. Before treatment, the animals were marked with picric acid and given seven days to get used to the laboratory environment. Toxic signs were monitored daily, including body mass, mortality, food and drink intake, and poisoning markers.

2.6 Haematological Investigations

A moderate ether sedative was administered after the animals had fasted for eighteen hours on the 29th day. After being drawn from the retro-orbital plexus, blood samples were placed in two tubes containing EDTA and the other without an anticoagulant for prompt analysis of haematological parameters. The second tube was used to separate the serum, which was centrifuged for 10 min at 4000 rpm and 4°C. It was thereafter stored at -20°C until its biochemical properties were investigated. Using potassium EDTA (1.5 mg/ml) for haematological analysis and sodium heparin (200 IU/ml) as an anticoagulant, blood samples were drawn from the (retro) ocular sinus. After that, the samples were centrifuged for ten minutes at 3000 rpm.

2.7 Biochemical Investigations

Blood samples were taken, and clotting time was allowed. The serum was isolated and kept at -20°C after a 10-min centrifugation at 3000 rpm to preserve its biochemical stability until analysis. In EC*'s* biochemical investigations, serum was utilised to evaluate several biochemical parameters. Blood samples from experimental and control rats were analysed for glucose level, Renal Function Test (RFT), Liver Function Test (LFT), and lipid profile using accepted procedures. The activity of serum alkaline phosphatase, glutamate pyruvate transaminase (SGPT/ALT), and glutamate oxaloacetate transaminase (SGOT/AST) was assessed using colorimetric methods. Specific wavelengths for these tests were 340 nm for SGOT/AST and SGPT/ALT, and 405 nm for alkaline phosphatase.

2.8 Urine Analysis

After the therapy period, the urine samples were taken. To gather 24 h urine samples, animals were placed in metabolic cages, which were used throughout the study, typically spanning several weeks. These cages allow for urine collection while preventing faecal contamination. During the collection period, animals had unrestricted access to water but were not given any food. Toluene was added as a preservative to the urine samples immediately after collection to prevent degradation. The urine was then centrifuged to remove any sediments and used for analysis.

2.9 Histopathological Examinations

Each animal had an autopsy, the weights of its liver, kidneys, and spleen were noted, and all animals were humanely euthanised on day 29. The control group and the cohort of animals given the highest dosage were subjected to histopathological examination. The group that received

the median dosage will also be analysed if anomalies are discovered in the highest-dose group. All of the animal's organs were gathered and preserved for 24 hours in 10% buffered neutral formalin. To eliminate absolute alcohol, each organ was sectioned into 5-6 µm slices, dehydrated using an automated method, and then cleaned in xylene. After three immersions in molten paraffin at 50°C, the cleaned samples were embedded and put into paraffin blocks using "L" molds. A microtome was used to segment the blocks, and haematoxylin-eosin was used to stain the resultant slides[8].

2.10 Statistical Analysis

Analysis tools included Dunnett's test, GraphPad version 7.0, one-way ANOVA, and information on changes in body weight, food and water consumption, haematology, and blood analysis. Every data point was condensed to satisfy the $p<0.05$ significance level.

3. Results

3.1 Acute Toxicity Assessment

Rats were administered dosages of EC up to 2000 mg throughout the investigation. There were no fatalities, notable changes in body weight, behavioural abnormalities, or indications of toxicity during the 14-day monitoring period. Water and food consumption were unaffected. The absence of side effects at this high dosage level suggests that EC has a significant margin of safety.

Observations at a single dose of 2000 mg/kg revealed that both groups' posture showed normal posture. Aggression, piloerection, grooming behaviour, and decreased motor activity were missing, although alertness was evident. Both grip strength and tactile sensitivity were observed. There was also no hypnosis, analgesia, tears (lacrimation), protruding eyes (exophthalmos), diarrhoea, tremors, convulsions, muscular spasms, catatonia, muscle relaxation, or analgesia. Nevertheless, there were no documented cases of fatality, and writhing and respiratory abnormalities were noted.

Ēlāti Cūraṇam's dose-finding experiment evaluated behavioural indicators of toxicity. Their observations showed similarities and differences when comparing the control and test groups. The test group had a propensity for a rise in body weight, but the control group stayed within normal levels. Posture remained normal in both groups. No convulsions or limb paralysis were observed in the test group. In addition to piloerection, defecation, sensitivity responses, locomotion,

Table 1. Variations in the rats' body weight (g) after exposure to EC

Dosage	Days		
	1	7	14
Control (2% Carboxy Methyl Cellulose (CMC)	270.1±5.7	272.7 ± 9.7	270.1 ± 2.1
EC 2000 mg/kg, p.o	260.3 ± 4.4	261.4 ± 7.1	260. 2± 6.0

All values are expressed in mean±S.D., n = 3, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 2. Daily water intake (mL) of rats exposed to EC

Dosage	Days		
	1	7	14
Control (2% Carboxy Methyl Cellulose (CMC)	60.0 ± 1.62	62.0 ± 1.10	69.1 ± 1.04
EC 2000 mg/kg, p. o	69.5 ± 1.04	69.5 ± 2.07	71.8 ± 2.04

All values are expressed in mean±S.D., n = 3, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 3. Food intake (grams per day) of rats exposed to EC

Dose	Days		
	1	7	14
Control (2% Carboxy Methyl Cellulose (CMC)	62.4 ± 1.54	62.2 ± 1.62	62.7 ± 4
EC 2000 mg/kg, p.o	64.0 ± 2.24	64.4 ± 2.1	64.6 ± 2.7

All values are expressed in mean±S.D., n = 3, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

muscle grip, and urination, body tone was the same in both groups. However, the test group did not exhibit lacrimation and salivation, but the control group did. Neither group showed any appreciable changes in skin tone. Both groups' somewhat noticeable rearing behaviour suggests that their answers were somewhat consistent. The test group showed significant variations in lacrimation, salivation, and body weight trends, even if many typical traits were shared.

3.2 Sub-Acute Repeated Oral Toxicity Study

EC showed good tolerance when given at modest doses of 100, 150, and 200 mg during 28 days. The absence of clinical signs, illness, or fatalities suggests that EC is well tolerated. The test groups experienced minimal weight fluctuations compared to the control group (Table 4). Furthermore, all animal groups' water and food intakes (Tables 5 and 6) were comparable and typical.

Ēlāti Cūraṇam (EC) demonstrated good drug tolerance in a 28-day Subacute Repeated Toxicology Trial by enduring daily dosages of 100, 150, and 200 mg/kg for 28 days without displaying any toxicity signs. No physical abnormalities or clinical signs of toxicity were seen for the whole dosage duration. All animals survived the study duration without observable distress. Body weight measurements showed minimal weight loss in all dosage groups, including the control group. The amount of food consumed by the control group and the various dosing groups was also normal and comparable.

3.3 Haematological and Biochemical Parameters

On the 28th day, after administering the drug multiple times, we evaluated the rat's haematological parameters to assess the drug's impact on blood components.

Table 4. Changes in body weight (g) exposed to EC

Dosage	Days				
	1	7	14	21	28
Control (2% Carboxy Methyl Cellulose (CMC)	180.6±3.62	181.4±4.14	183.7±9.61	184.6±3.03	185.7±1.31
100 mg/kg, p.o	183.2±1.14	182.7±3.64	180.4±8.32	180.1±4.66	178.4±3.76
150 mg/kg, p.o	186.6±1.64	184.3±2.74	182.4±8.32	181.1±3.16*	177.7±3.82**
200 mg/kg, p.o	184.4±6.74	182.6±2.12	178.6±2.36*	172.2±4.78	174.12±2.39**

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 5. Water intake millilitres per day for rat groups experiencing EC

Dosage	Days				
	1	7	14	21	28
Control (2% Carboxy Methyl Cellulose (CMC)	61.5±8.95	61.5±6.23	62.7±6.23	62.6±8.196	62.9±3.96
100 mg/kg, p.o	68.5±3.12	68.4±4.12	68.8±3.24	68.2±1.28	68.7±4.23
150 mg/kg, p.o	69.7±1.23	69.3±2.11**	69.1±1.13	69.4±1.21**	69.4±1.14
200 mg/kg, p.o	63.1±1.12	63.2±2.23**	63.4±1.23	63.2±2.33*	63.4±1.25**

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 6. Impact of EC on rat groups' food intake (g/day)

Dosage	Days				
	1	7	14	21	28
Carboxy Methyl Cellulose (CMC)	37.12±5.37	38.5±3.22	39.5±3.37	39.5±3.37	40±3.12
100 mg/kg, p.o	39.7±2.98	39.3±2.32	39.1±7.28*	40.4±2.92	41.6±1.62*
150 mg/kg, p.o	40.2±1.25	40.2±1.20	41.2±2.15	41.4±1.28	41.7±2.44**
200 mg/kg, p.o	41.3±1.24	41.6±1.24	42.7±2.66*	42.6±1.20*	42.1±3.12**

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 7. Effects of EC on the haematological parameters

Category	Control	100 mg	150 mg	200 mg
Haemoglobin	13.8±0.88	13.90±1.16	15.14±0.66	16.28±1.16
Total WBC (x10^3l)	11.91±0.5	11.85±1.23	11.08±1.21	11.11±2.27
Neutrophils (%)	33.65±0.06	33.3±1.24	32.11±2.16	33.20±1.10
Lymphocytes (%)	70.24±1.48	70.02±1.12	70.20±1.16	70.10±1.26
Monocytes (%)	0.86±0.07	0.58±0.19	0.72±0.13	0.71±0.60
Eosinophil (%)	0.54±0.09	0.54±0.12	0.62±0.16	0.72±0.04
Platelet cells 10^3/µl	687.17±8.76	678.71±9.16	675.18±2.20	672.16±3.74
Total RBC 10^6/µl	7.99±0.12	7.79±1.57	7.62±0.19	7.05±0.12
PCV%	37.79±0.6	37.35±1.23	37.98±1.18	36.82±2.14
MCHC g/dL	33.6±2.23	33.2±1.19	33.18±1.12	34.03±1.14
MCV fL(µm^3)	49.07±3.64	49.28±8.12	49.20±1.24	49.2±1.94

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 8. Effects of EC on biochemical parameters

Parameters	Control	100 mg/kg	150 mg/kg	200 mg/kg
Glucose (R) (mg/dl)	74.45±13.4	74.16±1.24	74.26±1.22	75.12±9.60
Total Cholesterol (mg/dl)	115.26±1.83	113.45±1.13	108.42±1.78	99.22±1.93
Triglycerides (mg/dl)	46.35±1.48	44.22±1.28	42.58±1.80	39.66±1.13*
Albumin(g/dl)	3.30±0.17	3.23±0.22	2.48±2.02	3.14±3.16
HDL	26.66±6.88	26.16±1.24	26.68±2.16	26.78±1.12
VLDL	15.20±2.44	15.82±1.14	15.44±2.14	15.24±4.16
LDL	73.80±2.43	70.24±3.14	68.14±1.24	65.64±4.12
pvalue	N.S	N.S	N.S	N.S

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 9. Renal function after EC treatment

Parameters	Control	100 mg/kg	150 mg/kg	200 mg/kg
Urea (mg/dl)	13.35±0.99	14.81±1.26	16.26±1.18	20.00±2.50
Uric acid (mg/dl)	5.37±0.35	5.11±0.43	6.72±2.15	6.00±0.20
Creatinine (mg/dl)	0.58±0.08	0.48±0.06	0.72±0.14	0.65±0.10
BUN (mg/dl)	15.12±0.10	15.12±0.28	16.28±0.14	17.50±1.20

All values are expressed in mean±S.D., n = 12, p<0.05, (ANOVA/Dunnett's test)

Table 10. Liver function after EC treatment

Parameters	Control	100 mg/kg	150 mg/kg	200 mg/kg
SGOT/AST(U/L)	114.95±1.39	115.15±2.11	116.21±1.23	116.55±1.23
T Bilirubin (mg/dl)	0.05±0.07	0.05±0.16	0.06±0.18	0.05±0.15
T. Protein (g/dl)	6.32±0.38	6.48±1.34	6.52±0.23	6.53±1.26
ALP(U/L)	146.25±8.77	144.2±6.27	149.16±4.17*	153.3±4.25*
SGPT/ALT(U/L)	71.23±1.28	72.91±1.59	72.34±2.18	71.32±1.28

All values are expressed in mean±S.D., n = 12, **(p<0.01), *(p<0.05), (ANOVA/Dunnett's test)

Table 7 illustrates the minor differences in haematological parameters between each treatment group and the corresponding control groups. Nonetheless, no noticeable physical changes occurred while administering the dosage. The analysis of biochemical data (Tables 8-10) revealed that total bilirubin, total protein, HDL, VLDL, SGOT, SGPT, urea, and creatinine levels remained within normal limits in comparison to the control group values.

3.4 Histopathology

A microscope fitted with a 100-x objective lens was used to examine the organs of the control and treatment groups. Following the administration of the test medication, EC, there were no signs of oedema, atrophy, or hypertrophy in the essential organs. These results demonstrate that the medication is risk-free and safe for long-term usage.

4. Discussion

Research in toxicology is the foundation for identifying possible risks throughout the safety evaluation procedure[9]. Determining the harmful effects and defining test compounds requires toxicity testing. Human toxicity is as common and severe as that shown in animal research. Animals are expected to be used in toxicity studies for some time to come due to the advantages of studying a whole, functioning organism[10]. Toxicology studies are essential to identifying risks in safety assessments. On the toxicity scale, a dosage of 2000 mg was categorised as Unclassified under OECD guideline 425[11]. Acute toxicity research was carried out utilising a dosage of 2000 mg/kg administered to animals to evaluate the potential dangers of EC when ingested. Additionally, a subacute oral toxicity study examined the effects of EC at various concentrations of 100, 150, and 200 mg/kg.

Food intake and body weight fluctuations are frequently employed to predict the harmful consequences of exogenous substances[12]. The ratiometric changes in these organs highlighted the significance of the liver and kidneys in drug metabolism and excretion compared to the body weight of treated rats[13]. One of the main causes of hypertension is body fat. Blood pressure frequently drops for people with obesity and high blood pressure when they use techniques including eating a healthy diet, exercising often, changing their behaviour, having bariatric surgery, or using anti-obesity drugs. Blood pressure readings improve more when weight reduction is more substantial. Losing extra weight reduces the risk of uncontrolled blood pressure in overweight or obese individuals with hypertension. On the other hand, gaining weight is significantly associated with a higher risk of poorly managed hypertension[14,15]. Body weight was constant throughout the trial, as seen in Table 1. Dose-dependent changes in body weight are seen in Table 4, where decreases become more pronounced at high dosages (150 and 200 mg/kg). From the 14th day onwards, there were noticeable drops, especially in the 200 mg/kg group, which may indicate systemic or metabolic effects at higher dosages. On the other hand, the lower-dose group (100 mg/kg) showed comparatively steady body weight, suggesting improved tolerance at this dosage. Similarly, food intake data in Table 3 revealed no significant changes, with both groups maintaining stable consumption patterns. This consistency supports the conclusion that EC at the administered dose does not negatively impact appetite, gastrointestinal function, or metabolic processes. These findings collectively indicate that EC at 2000 mg/kg is well tolerated and does not disrupt key physiological functions in rats. Statistical analyses, including ANOVA and Dunnett's test, confirmed the reliability of the observed trends. The risk of hypertension may be reduced by consuming 6–8 cups of plain water daily. This implies that consuming enough water may positively influence blood pressure control[16]. Table 2 shows that all groups' daily water consumption was constant, with only modest deviations within acceptable physiological limits. This indicates that 2000 mg/kg EC does not disrupt hydration or induce stress responses.

Haematological and clinical chemistry parameters are trustworthy markers for determining toxicity[17]. Haematological evaluations are commonly employed to ascertain the toxicity caused by various chemicals, including plant extracts and pharmaceuticals. By looking at haematological parameters, researchers may determine how a chemical influences the body's hematopoietic and immunological systems. This kind of study sheds light on the safety profile of the compounds under examination and aids in identifying any hazardous effects. Essential information on the body's reactions to stress, trauma, lesions, and deprivation is frequently provided by blood profiles[18]. The production of blood cells is referred to as haematopoiesis. Extrapolating from animal research, changes in the hematopoietic system increase the prediction accuracy for human toxicity[19]. As shown in Table 7, the test drug demonstrated a dose-dependent

increase in haemoglobin levels, with the highest value recorded at 200 mg/kg (16.28±1.16), suggesting a potential erythropoietic or hematinic effect. Total White Blood Cell (WBC) counts remained within normal limits across all groups, with no significant deviation from the control, indicating the absence of leucocytosis or leukopenia. Similarly, differential leukocyte counts—including neutrophils, lymphocytes, monocytes, and eosinophils—remained stable, suggesting that the immune response and inflammatory status were unaffected by the treatment. Platelet counts showed a slight, non-significant decrease with increasing doses but stayed within physiological limits, implying no detrimental effect on thrombopoiesis. Although a gradual reduction in Red Blood Cell (RBC) count was observed, particularly at 200 mg/kg, the simultaneous rise in haemoglobin levels suggests enhanced haemoglobin synthesis or improved haemoglobin content per RBC. Packed Cell Volume (PCV) showed only minor fluctuations, with no significant alterations. Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) remained consistent, supporting the stability of red cell morphology and haemoglobin concentration. Overall, the test drug appears to exert a favourable influence on haemoglobin levels without compromising other haematological parameters. The stability of WBC counts, differential leukocyte profiles, platelet numbers, and red cell indices across the treatment groups supports the haematological safety of the formulation. Notably, the increased haemoglobin levels at higher doses highlight the potential therapeutic utility of the formulation in the management of anaemia.

The biochemical analysis of the glucose and lipid profiles in each treatment group did not demonstrate any statistically significant changes in comparison to the control group, as indicated in Table 8. All test drug doses did not affect basal glucose metabolism, as evidenced by the fact that fasting glucose levels stayed constant. With the lowest value seen at 200 mg/kg, total cholesterol levels showed a dose-dependent decline, indicating a possible cholesterol-lowering action. With higher dosages, triglyceride levels also gradually decreased, reaching a statistically significant drop at 200 mg/kg ($p<0.05$), suggesting a potential impact on lipid metabolism. Although there was significant fluctuation, albumin levels did not exhibit any continuous pattern or statistical significance, remaining within acceptable physiological limits. All things considered, the test medication seems to have a little hypolipidemic impact without appreciably changing important lipid fractions or glucose metabolism. It is crucial to consider how hypercholesterolemia and arterial hypertension frequently coexist, speeding up the evolution of atherosclerosis, given the relationship between cholesterol levels, blood pressure responses to adrenergic stimulation, and the development of target organ damage in hypertension. Investigating the connections between these disorders may help clarify the advantages of treatments that address both high blood pressure and high cholesterol[20].

The renal safety profile of the test drug was assessed through both biochemical parameters and histopathological analysis. As presented in Table 9, a dose-dependent increase in serum urea levels was observed, with the highest value recorded at 200 mg/kg (20.00±2.50 mg/dL). Although this value remains within the generally acceptable physiological range, the upward trend suggests the need for careful monitoring at higher doses to assess potential effects on nitrogen metabolism or renal excretory function. Uric acid levels showed slight, non-significant fluctuations across the groups, peaking at 150 mg/kg (6.72±2.15 mg/dL) and declining at 200 mg/kg. This variation is likely attributable to individual metabolic differences rather than a consistent drug-related effect. Serum creatinine levels remained relatively stable across all groups, with only minor increases at the higher doses, indicating preserved glomerular filtration and overall kidney function. Similarly, Blood Urea Nitrogen (BUN) levels exhibited a slight dose-related rise, reaching 17.50±1.20 mg/dL at 200 mg/kg, mirroring the serum urea trend and potentially reflecting a mild increase in renal workload or alterations in protein metabolism. Importantly, histopathological examination of kidney tissues revealed no evidence of tissue injury or structural abnormalities, further confirming the absence of nephrotoxicity.

Important information on the structural and functional integrity of the liver may be obtained from serum liver function tests. The liver plays an important role in the manufacturing of proteins; hence, a healthy liver may provide hypertensinogen, a globulin required for blood pressure regulation. When liver function is impaired, however, the liver's capacity to produce hypertensinogen is reduced. Because of this, disorders linked to abnormal Liver Function Test (LFT) findings are more likely to co-occur with problems like hypertension, highlighting the need for liver health maintenance[21]. The test drug's

hepatological analysis showed no signs of toxicity in any of the treatment groups. Table 10 demonstrates that there was no hepatocellular damage or hepatic enzyme leakage as the blood levels of SGOT (AST) and SGPT (ALT) were constant and comparable to the control group. Additionally, there were no discernible changes in total bilirubin levels, indicating that hepatic excretory function and bilirubin metabolism were unchanged. Although the treated groups' total protein levels increased somewhat, they remained within normal physiological bounds, which may indicate a little supporting effect on the liver's synthesis ability.

Albumin and total protein levels show the liver's overall synthesis performance, whereas Alkaline Phosphatase (ALP) is a measure of biliary function. Hepatocellular integrity is evaluated by aminotransferases like ALT and AST. ALT is thought to be the most liver-specific marker because of its restricted distribution in contrast to AST, which is also seen in the kidneys, testes, heart, and muscles. The dosages of 150 mg/kg and 200 mg/kg resulted in a statistically significant ($p<0.05$) increase in ALP levels. The lack of comparable elevations in ALT, AST, and bilirubin levels indicates that high ALP may be a healthy or adaptive response rather than a sign of liver disease, even if it can be connected to hepatic or bone activity[22]. The biochemical results were further supported by histological examination of liver tissues, which showed no indications of pathological changes or structural damage. Together, these findings confirm that there is no indication of any negative effects on liver function and that the test formulation is hepatologically safe at all tested dosages.

Histological examination of tissues and organs is essential for validating clinical and physical findings and offering conclusive information on the existence or lack of toxicity. From minor alterations like congestion and degeneration to more serious, irreversible harm like necrosis, histopathological lesions can vary widely. Necrosis represents terminal, permanent cell destruction, whereas degenerative alterations usually signify reversible cellular harm[23]. When assessing systemic toxicity, the liver and kidneys—two essential organs in

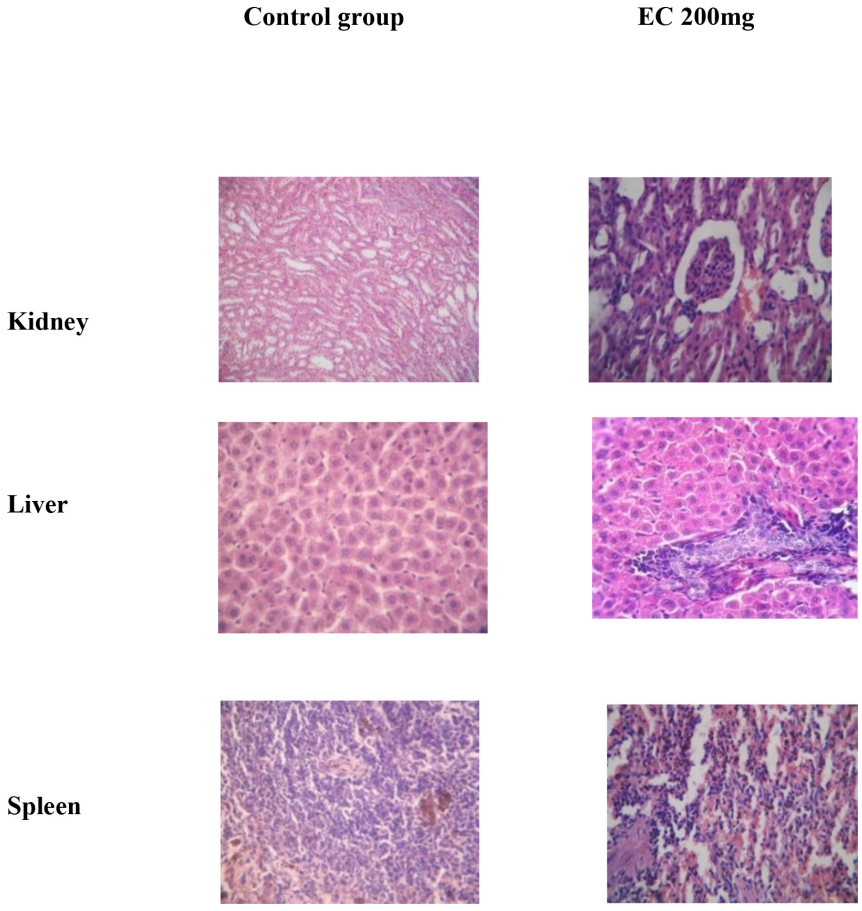


Figure 1. Histopathological findings in test drug-treated groups.

charge of metabolism, detoxification, and excretion—are particularly crucial. Histopathological analysis of the major organs in this study showed intact tissue architecture, suggesting that EC had no harmful effects. There were no indications of degradation, and the neural structures were still intact. There was no sign of necrosis or inflammatory infiltration, and the liver parenchyma looked normal, as seen in Figure 1. There were no indications of atrophy or pathological alterations, and the kidneys' proximal and distal convoluted tubules were in good condition. Additionally, there were no indications of necrosis or atrophy, and the spleen maintained its usual structure. Overall, these histopathological results imply that at the measured dosages, EC does not appear to do any harm to important organs. The conclusion that the formulation is safe and non-toxic in the circumstances of this study is supported by the lack of inflammation, infiltration, or structural anomalies.

5. Conclusion

The trial drug EC was deemed safe even at high doses. Further assessments of repeated oral toxicity at various doses showed no significant adverse effects. Consequently, based on the research, EC has not been shown to harm vital organs. Research has also shown that standard doses of EC do not cause significant toxicological effects on specific organs, biomedical indicators, or haematological parameters in Wistar albino rats during the study. The polyherbal formulation of EC seems to be safe for extended use.

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