INVESTIGATION OF EXTRACT FROM *JASMINUM SUBTRIPLINERVE* BLUME LEAVES FOR ACUTE AND SUBCHRONIC ORAL TOXICITY IN EXPERIMENTAL ANIMALS

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This research is conducted to evaluate the acute and subchronic toxicities of the extract of Jasminum subtriplinvere Blume leaves through oral administration in experimental animals. The acute toxicity was determined by the Litchfield Wilcoxon method in Swiss mice. The subchronic toxicity was evaluated by WHO and OECD's recommendation in Wistar rats with oral doses of 2.4 g/kg/day and 7.2 g/kg/day for 90 consecutive days. We found no sign of toxicity and no mortality was observed in Jasminum subtriplinvere Blume treated mice at 5000 mg/kg. In terms of the subchronic toxicity test, after oral administration of the extract of Jasminum subtriplinvere Blume, hematological parameters, hepato-renal functions, and microscopic images of the liver and kidney were unchanged as compared with the control group. In conclusion, the extract of Jasminum subtriplinvere Blume leaves did not produce acute and subchronic toxicities in Swiss mice and Wistar rats.

Keywords: Jasminum subtriplinvere Blume, acute toxicity, subchronic toxicity, experimental animals.

I. INTRODUCTION

Nature has been a source of medicinal agents from ancient times, and medicinal plants formed a wide variety of traditional medicines used in various countries worldwide.[1]

The exclusive use of herbal drugs for managing various ailments continues due to easy access, better compatibility, and economic reasons. According to the World Health Organization (WHO), up to 80% of developing country populations use traditional medicine for primary health care. However, the lack of evidence-based approaches and lack of toxicological profiling of herbal preparations caused the biggest concern of medicinal plant use. Thus, evaluating their toxicity plays a vital role in recognizing these effects, supporting their characterization, evaluating human risk, and proposing measures to mitigate the risk, particularly in early clinical trials.[2] Toxicity refers to the unwanted impacts on biological systems. To evaluate biological toxicity, it is crucial to choose the correct system since no effect may otherwise be seen. Toxicity of a substance can be impacted by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection), the time of exposure (brief, acute, subchronic, or chronic exposure), the number of exposures (a single dose or multiple doses), the physical form of the toxin (solid, liquid, or gas), the organ system involved (cardiovascular, nephro-, hemo-, nervous-, or hematopoietic-system), and even the genetic

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makeup and robustness of the target cells or organisms.[3]

Subchronic systemic toxicity is defined as adverse effects occurring after repeated or continuous administration of a test sample for up to 12 weeks or not exceeding 10% of the animal's lifespan.[4,5]

Jasminum subtriplinerve Blume, a species of Oleaceae, is widely distributed worldwide, growing naturally in the mountains and midlands of countries such as India, Myanmar, Cambodia, Laos, and the southern provinces of China.[6] In Vietnam, *J. subtriplinerve* can be found in numerous provinces across mountains, midlands, and plains, including Lao Cai, Hoa Binh, Hanoi, Nghe An, Ha Tinh, Quang Tri, and Thua Thien Hue.[7] Traditional medicine employs *J. subtriplinerve* leaves, which are primarily utilized to manage irregular menstruation in postpartum women with high fever, lymphadenitis, mastitis, breast abscess, leukorrhea, rheumatism-induced bone and joint pain, scabies, impetigo, and pruritic skin conditions.[8] The plant serves various medicinal purposes, such as supporting liver function, promoting bile secretion, aiding digestion, improving appetite, facilitating sleep, and demonstrating antibacterial and anti-inflammatory properties.[9] Investigations into chemical composition and biological activities reveal that *J. subtriplinerve* leaves contain phenylethanoid glycosides (e.g., verbascoside), terpenoids, flavonoids (e.g., kaempferol), phenolic acids (e.g., p- hydroxybenzoic acid, protocatechuic acid), and steroids (e.g., β-sitosterol).[10] These constituents contribute to the plant's antibacterial, anti-inflammatory, antioxidant, liver-protective, cytotoxic, and antipyretic effects.[6,10-14]

Historically, these natural products have been used since ancient times and in folklore to treat many diseases and illnesses. Previously, our research team evaluated sub-chronic toxicity with two dosage levels of 18 and 54 mg/kg/day,[15] however, there has been no report available on the safety of dosages 2.4 g/kg/day and 7.2 g/kg/day. Therefore, we intended to investigate the acute and subchronic toxicities of leaf extract of *Jasminum subtriplinvere* Blume. with two dosages of 2.4 g/kg/day and 7.2 g/kg/day in animals.

II. MATERIALS AND METHODS

1. Preparation of extract *Jasminum subtriplinvere* Blume leaves

Fresh tea leaves bought in Nghe An are cleaned and dehydrated, then extracted twice with a ratio of 1:4 (1kg of leaves, 4 liters of water) at 100 degrees Celsius, under normal pressure for 10 minutes. Extraction for 12 hours will produce 1g of extract in a solid form.

2. Experimental animals

Wistar rats (180 ± 30g) and *Swiss* mice (20 ± 2g) were used in this study. The animals were housed in cages (groups of ten rats or mice/cage) in a room with access to a standard certified rodent diet and water ad libitum. They were acclimated to housing for 5 - 7 days before the experiment at the Department of Pharmacology, Hanoi Medical University.

Acute toxicity study

The acute toxicity study was carried out according to WHO Guidance (World Health Organization (2000)) and OECD 423 guidelines.[16,17]

Following the overnight fasting period, a total of 30 mice were randomly assigned to the study. The test extracts were administered at dosages of 300, 2000, and 5000 mg/kg body weight (bw) (n = 10). The gavage volume of 0.2 mL/10 g body weight using stomach tubes was adjusted according to the weight of each

mouse. The animals were closely observed individually for the first 30 min for any signs of acute toxicity and behavioral changes, then for 4 hours, and then at least once daily for 14 days. The starting dose of 5000 mg/kg was selected based on the literature of Do Tat Loi for the human dose of *Jasminum subtriplinvere* in folk medicine. Ten animals were used for each step with a 3-day interval between doses to allow for the observation of delayed toxicity before administering the next dose level.[17]

Subchronic toxicity study

Subchronic toxicity study was carried out according to WHO Guidance and OECD 408 guidelines.[16,18]

The study was carried out in the course of continuous 90 days. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control group) was given an administration of distilled water;

- Group 2: Group was administered orally "extract of *Jasminum subtriplinvere* Blume leaves." at 2.4 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3: Group was administered orally "extract of *Jasminum subtriplinvere* Blume leaves" at 7.2 g/kg/day (3 times as high as the dose at group 2). Animals were given the oral administration of distilled water and extract of *Jasminum subtriplinvere* Blume leaves at 10 mL/kg b.w daily for consecutive 90 days and observed once daily to detect clinical signs and time points for laboratory tests. The extract was dissolved in distilled water daily before giving orally to rats.

The signs and parameters were checked during the study including general conditions, mortality, and clinical signs.

- Weekly assessment of general health status, body weight

- Monitoring of hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, mean corpuscular volume (MCV), total white blood cells (WBC), WBC differentials, platelet count (PLT).

- Serum biochemistry test: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, total cholesterol and creatinine levels. The aforementioned parameters were checked before treatment and at 30 days, 60 days, and 90 days after treatment. At the end of the experiment, all animals were subjected to a full gross necropsy. The livers and kidneys of 30% rats of each group were harvested for histopathology examinations. The micro-histological examination was carried out at the Center for Research and Early Detection of Cancer (CREDCA).

3. Statistical analysis

Data sets were entered and analyzed using Excel 2013 software. Results were expressed as the Mean value ± Standard Deviation (SD) or the percentage (%). The level of significance was considered at values of $p < 0.05$. The two arms of the recovery group were analyzed by the Student's t-test. Unless otherwise noted, 'significant' means that it has statistical significance compared with the control group.

III. RESULTS

1. Acute toxicity study

In the acute toxicity study, oral administration of a single dose of up to 5000 mg/kg did not exhibit any mortality nor signs of toxicity during the observational period of 14 days. No significant change in the body weight gains were detected. Therefore, the approximate lethal dose (LD_{50}) of *Jasminum subtriplinvere* Blume extract was estimated to be higher than 5000 mg/kg. Based on the Globally Harmonized

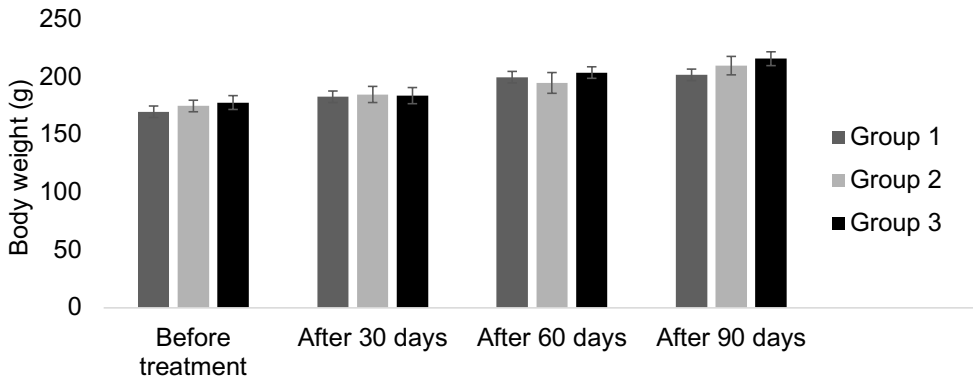
System of Classification and Labeling of Chemicals, the substances having an LD50 value greater than 5000 mg/kg are considered as unlikely to present acute hazard.

2. Subchronic toxicity study

General condition and body weight changes

Animals had normal locomotor activities and goods feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes.

Figure 1 showed that after 30 days, 60 days, and 90 days, the body weight of all groups increased as compared with before treatment ($p<0,05$). There was no significant difference between the treated groups and the control group ($p>0,05$).



* $p < 0.05$ as compared with the time point "Before treatment"

Δ $p < 0.05$ as compared with the control group (Group 1)

Figure 1. The effect of Jasminum subtriplinvere Blume extract on body weight changes

3 . Effect of *Jasminum subtriplinvere* Blume extract on hematopoietic function

Table 1. The effect of *Jasminum subtriplinvere* Blume extract on hematopoietic function

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Red blood cells count (T/L)	Group 1	10.50 ± 0.61	10.43 ± 0.82	10.13 ± 0.78	10.35 ± 0.92
	Group 2	10.14 ± 0.85	10.93 ± 0.63	10.31 ± 0.88	10.51 ± 0.90
	Group 3	10.78 ± 0.69	11.01 ± 0.49	10.91 ± 0.73	10.75 ± 0.87
	p	> 0.05	> 0.05	> 0.05	> 0.05
Hemoglobin level (g/dL)	Group 1	13.59 ± 0.98	13.21 ± 1.47	13.05 ±1.63	13.19 ± 1.21
	Group 2	13.06 ± 1.54	14.48 ± 1.01	13.81 ± 1.17	14.05 ± 1.65
	Group 3	14.90 ± 1.94	14.98 ± 1.42	14.92 ± 1.72	14.25 ± 1.16
	p	> 0.05	> 0.05	> 0.05	> 0.05

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Hematocrit (%)	Group 1	46.21 ± 2.06	46.19 ± 3.71	46.21 ± 4.59	43.70 ± 2.36
	Group 2	46.58 ± 2.89	45.76 ± 2.26	45.33 ± 3.06	42.47 ± 5.21
	Group 3	43.76 ± 4.59	43.63 ± 1.85	43.69 ± 2.93	42.06 ± 4.54
	p	> 0.05	> 0.05	> 0.05	> 0.05
Platelet count (G/L)	Group 1	551.76 ± 78.03	574.19 ± 81.03	542.84 ± 75.26	520.75 ± 90.27
	Group 2	569.93 ± 80.93	586.15 ± 101.12	527.84 ± 68.04	553.57 ± 81.04
	Group 3	548.76 ± 97.33	521.56 ± 79.45	530.68 ± 68.92	564.36 ±78.92
	p	> 0.05	> 0.05	> 0.05	> 0.05
Total WBC count (G/L)	Group 1	8.19 ± 1.71	9.02 ± 1.51	8.40 ± 1.18	7.99 ± 1.25
	Group 2	8.06 ± 1.53	8.37 ± 1.19	8.93 ± 2.01	9.12 ± 1.45
	Group 3	8.75 ± 1.37	8.05 ± 1.54	8.32 ± 1.43	8.95 ± 1.25
	p	> 0.05	> 0.05	> 0.05	> 0.05
Lymphocytes (%)	Group 1	76.92 ± 6.93	71.05 ± 9.78	74.65 ± 8.06	70.19 ± 7.05
	Group 2	75.14 ± 7.38	76.92 ± 8.32	74.24 ± 9.09	77.26 ± 9.50
	Group 3	74.13 ± 6.98	75.53 ± 8.41	78.04 ± 8.52	82.84 ± 7.85
	p	> 0.05	> 0.05	> 0.05	> 0.05
Neutrophils (%)	Group 1	15.21 ± 4.04	17.31 ± 4.23	14.92 ± 4.01	17.17 ± 5.28
	Group 2	15.89 ± 4.54	15.12 ± 4.98	15.15 ± 4.82	14.02 ± 4.20
	Group 3	14.99 ± 4.27	16.06 ± 4.88	14.51 ± 3.98	12.97 ± 3.76
	p	> 0.05	> 0.05	> 0.05	> 0.05

p: compared with the control group and the time point "Before treament"

There were no significant differences red blood cell count, hematocrit, hemoglobin level, platelet count, total WBC count, and WBC between the treated groups and the control group (p>0.05).

4. Effect of *Jasminum subtriplinvere* Blume extract on liver

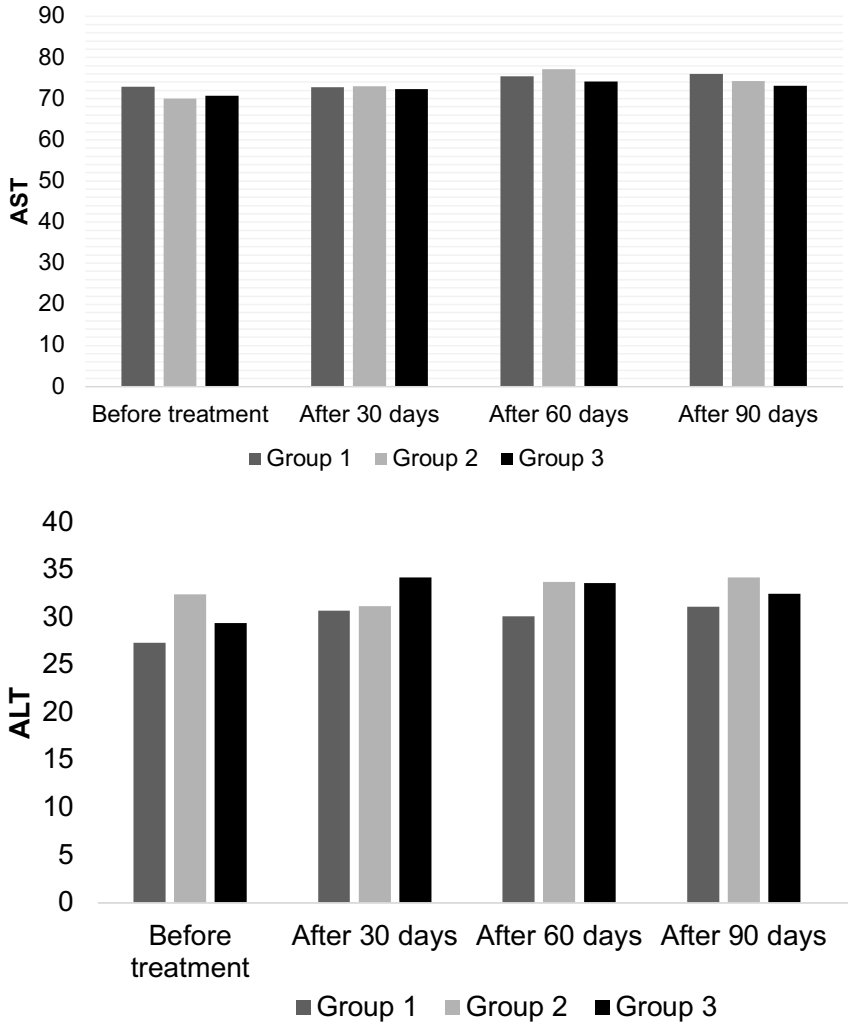


Figure 2. Effect of *Jasminum subtriplinvere Blume* Extract on AST and ALT level

Figure 2 demonstrates that after 30 days, 60 days, and 90 days of treatment, extract of *Jasminum subtriplinvere* Blume at 2.4 g/kg/day and 7.2 g/kg/day did not cause statistical difference in AST and ALT levels when comparing the treated groups to the control group (p>0.05).

5. Effect of *Jasminum subtriplinvere* Blume extract on liver function

Table 2 illustrates that after 30 days, 60 days, and 90 days of treatment, there was no statistical difference in total bilirubin, albumin, and total cholesterol concentration in all treated groups compared to the control group (p>0.05).

Table 2. The effect of leaves of *Jasminum subtriplinvere* Blume on liver function

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Total billirubin (mmol/L)	Group 1	51.2 ± 6.74	48.90 ± 8.91	58.31 ± 15.51	59.80 ± 11.32
	Group 2	57.10 ± 15.65	47.85 ± 7.86	57.91 ± 12.89	71.10 ± 18.03
	Group 3	51.31 ± 9.03	46.08 ± 10.21	54.30 ± 12.67	58.50 ± 10.04
Albumin concentration (g/dl)	Group 1	2.91 ± 0.45	3.14 ± 0.33	2.75 ± 0.52	3.01 ± 0.67
	Group 2	2.90 ± 0.39	3.31 ± 0.65	2.69 ± 0.30	3.12 ± 0.51
	Group 3	2.92 ± 0.34	3.32 ± 0.60	2.69 ± 0.51	2.89 ± 0.54
Total cholesterol concentration (mmol/L)	Group 1	1.51 ± 0.21	1.69 ± 0.26	1.38 ± 0.28	1.60 ± 0.32
	Group 2	1.53 ± 0.31	1.78 ± 0.39	1.46 ± 0.19	1.68 ± 0.33
	Group 3	1.55 ± 0.24	1.83 ± 0.31	1.59 ± 0.29	1.71 ± 0.41

6. Effect of extract of leaves of *Jasminum subtriplinvere* Blume. on kidney function

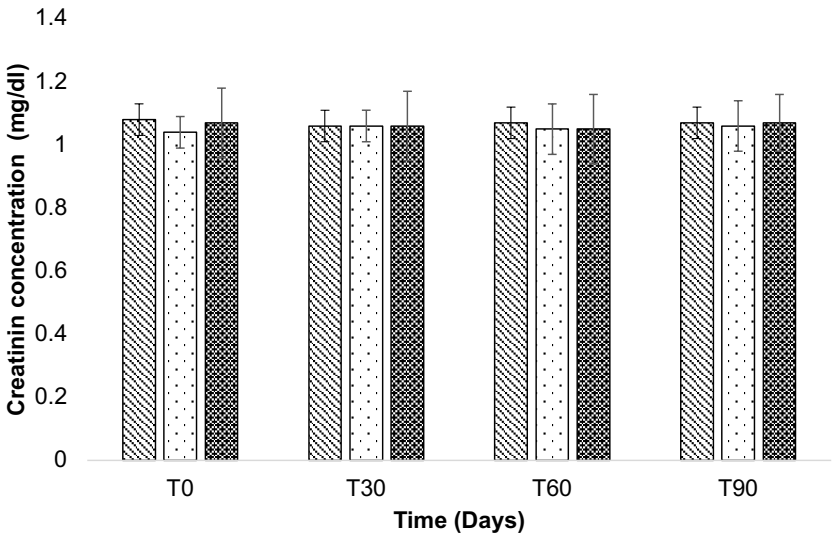


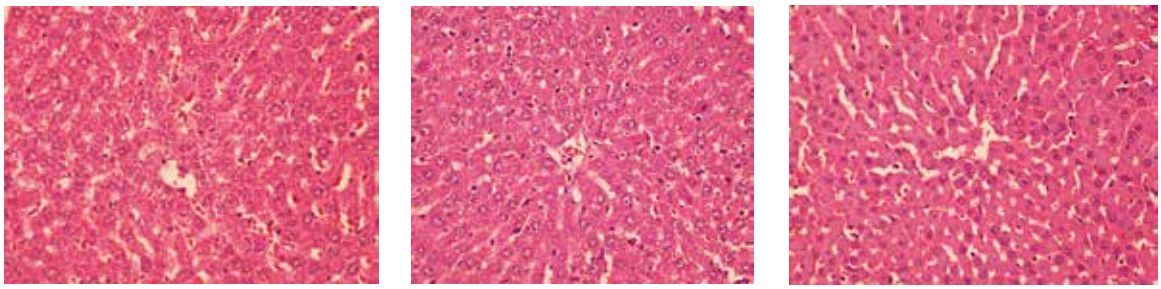
Figure 3. Effect of Jasminum subtriplinvere Blume extract on creatinin level

Figure 3 demonstrated that after 30 days, 60 days, and 90 days of treatment, extract of *Jasminum subtriplinvere* Blumecaused no significant difference in serum creatinine levels between the control group and the two treated groups ($p > 0.05$).

7. Histopathological examination

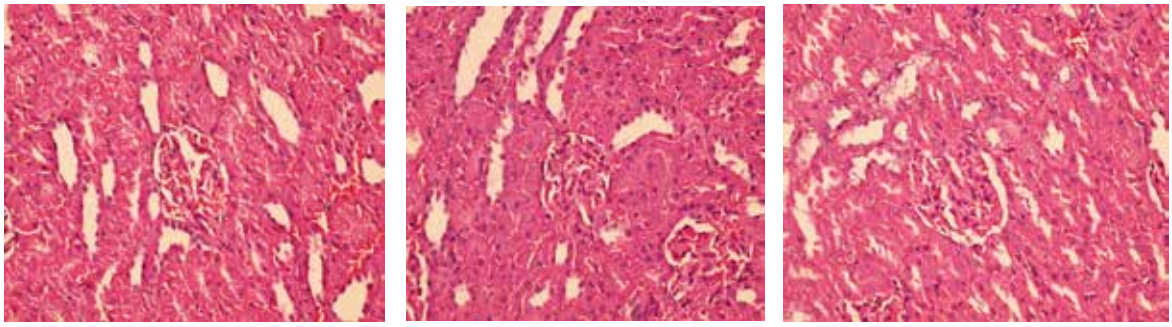
No gross lesion or change in size were observed when all experimental rats were subjected to a full gross necropsy which examined the hearts, livers, lungs, kidneys, and abdominal cavities.

There was no significant difference in histopathological examinations of livers and kidneys between treated mice and the control group after 90 days (Figures 4 and 5).



Group 1 Group 2 Group 3

Figure 5. Histopathological morphology of liver (HE × 400)



Group 1 Group 2 Group 3

Figure 6. Histopathological morphology of kidney (HE × 400)

IV. DISCUSSION

1. Acute toxicity of *Jasminum subtriplinvere* Blume extract.

In this experiment, the acute oral toxicity test showed that *Jasminum subtriplinvere* Blume Extract was tolerated up to 5000mg/kg. Moreover, no sign of toxicity and no mortality were observed for seven days continuously. As a result, oral LD50 of *Jasminum subtriplinvere* Blume extract was not determined in mice. As defined by WHO, *Jasminum subtriplinvere* Blume extract was a safe herbal medicine. According to Nguyen Tra My (2023), seven compounds, including verbascoside (1), p-hydroxybenzoic acid (2), protocatechuic acid (3), vanillic acid (4), kaempferol (5), quercetin (6), and β-sitosterol (7) were isolated from the extract of *J. subtriplinerve.* Furthermore, verbascoside (1) is a major compound with levels of *J. subtriplinerve.*[19] Nowadays, the world has not reported studies on the acute toxicity of the extract of *J. subtriplinerve*. Besides, Jefferson Gustavo Henn (2023) indicated that cytotoxicity assays demonstrated that verbascoside reduced cell viability only at the highest concentrations, and verbascoside had no phototoxic properties, the in vivo toxicity evaluation of verbascoside suggested that the LD50 is higher than 5000 mg/Kg.[20]

2. Subchronic toxicity of *Jasminum subtriplinvere* Blume extract.

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is important to choose the correct system since no effect may otherwise be seen. The toxicity of a substance can be impacted by many factors, such as the route and time of

exposure, the physical form of the toxin, and the organ system involved. The subchronic study provides information on the undesirable effects of continuous or repeated exposure to plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents.

Body weight changes are the most basic index to reflect toxicity to organs and systems and reflect the combined effects of xenobiotics on the body.[21] For all experimental animals, general signs should be observed daily, and body weight should be measured periodically.[22] It can be stated that extract of *Jasminum subtriplinvere* Blume did not interfere with the animals' normal metabolism as corroborated by the non-significant difference from animals using the distilled water as the control group.

The blood circulatory system performs essential functions, such as delivering oxygen to all body tissues, maintaining vascular integrity, and providing necessary immune factors for host defense reaction. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an essential parameter for humans and animals' physiological and pathological status.[22,23] Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies. After 30 days, 60 days and 90 days of the treatment, there was no significant difference in total red blood cells, hematocrit, hemoglobin level, platelet count, total WBC count, and WBC differentials between the treated groups and the control group, so it can be concluded that *Jasminum subtriplinvere* Blume extract does not affect the hematological system.

Analysis of the kidney and liver is critical in the toxicity evaluation of drugs and plant extracts as they are both necessary for an organism's survival. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.[24] The changes in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) contents are a sensitive index to reflect the degree of liver cell damage. When chronic liver injury happens, AST and ALT are released from the liver cells' injury, increasing in the serum.[17] Creatinine levels can be used in describing the function of the kidneys.[18] There are no significant ALT and AST changes in both male and female rats at all doses, which indicates that extract of *Jasminum subtriplinvere* Blume had no deleterious effect on liver function. The blood biochemistry level of control and extract of *Jasminum subtriplinvere* Blume in treated rats at various doses presented no significant difference between the treated and the control groups ($p > 0.05$). This evidence shows that extract of *Jasminum subtriplinvere* Blume did not affect the liver and kidney functions.

In various organs, the liver and kidney are vital for the drug's affinity and conducive to eliminating the drug and having a particular role in the accumulation. The histopathological examination revealed the alteration in cell structure under the light microscope.[20] Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of the extract of *Jasminum subtriplinvere* Blume. Our study showed no significant difference in histopathological examinations of the livers and kidneys between the treated groups and the control group.

Overall, this study's findings indicated that no significant difference was observed in blood parameters, biochemistry parameters, and histopathological observations of liver and

kidney tissues between the reated groups and the control group. Currently, worldwide there are not many studies on the toxicity of this medicinal herb; several plants in the *Jasminum* family have also been studied for similar toxicity, such as *Jasminum sambac* L., according to Phanukit Kunhanchan (2012), the oral administration at a dose of 5,000 mg/kg did not produce acute oral toxicity.[25] Results from Siti Balkis Budin (2012), *L. elliptica did not* produce acute oral toxicity at doses from 400 to 5000 mg/kg; at 125, 250, and 500 mg/kg administered for 28 consecutive days, no alteration in body weight, and food and water consumption were noted.[26] The hematological and biochemical analyses did not show significant difference between control and treated groups in most of the parameters examined. According to Tuoi Thi Hong Do (2022), the 50% ethanolic extract of *J. subtriplinerve* did not cause any toxic sign in mice at the Dmax of 20 g/kg.[27] According to Nguyen Tra My (2023),[19] verbascoside is a major compound with levels of J. subtriplinerve and this compound was also shown to have no sign of toxicity found in subchronic exposure.[20]

V. CONCLUSION

No sign of toxicity and no mortality was observed in treated mice at the dose of 5000 mg/kg. Oral LD50 of the extract of *Jasminum subtriplinvere* Blume leaves was not determined in *Swis*s mice.

For continuous 90 days, the extract of *Jasminum subtriplinvere* Blume leaves. at oral doses 2.4 g/kg/day and 7.2 g/kg/day did not create any toxic sign or symptom of subchronic toxicity in *Wistar* rats.

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Declaration of competing interest

We have no conflict to declare.

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