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Subacute Oral Toxicity Study of a New Polyherbal Formulation of Usadha on liver and Kidney Function of Wistar Rats

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Usadha is a traditional Balinese medicine system that has long been used in ancient medicine systems. However, so far there have been no reports on its safety. This study aims to examine the effect of polyherbal Usadha on hematology and blood biochemistry of Wistar rats. The study used a Completely Randomized Design with six treatments, namely polyherbal Usadha supplementation with doses of 0 mg/kg bw/day (E0), 100 mg/kg bw/day (E1), 200 mg/kg bw/day (E2), 300 mg/kg bw/day (E3), 400 mg/kg bw/day (E4), and 500 mg/kg bw/day (E5). The treatments were given for four weeks. The variables measured were complete hematology and blood biochemistry. Data were analyzed by One-Way ANOVA and continued with the Least Significant Difference test at a 95% confidence interval. Polyherbal Usadha appears to be safe for consumption without significant risk of side effects on blood parameters and liver function. Additional studies including histological analysis and mechanism of action will provide deeper insight into the therapeutic effects of polyherbal Usadha. In conclusion, polyherbal usada is relatively safe for liver and kidney function in Wistar rats. The results of this study support the safety of using Poliherbal Usadha as a candidate herbal medicine for long-term consumption.

Keywords: Blood biochemistry; Citrus amblycarpa (Hassk.) Ochse; Haematology; Piper nigrum L; Polyherbal Usadha.

Traditional medicine is gaining popularity because it supplies phytopharmaceuticals for the development of pharmacological medicines with desired qualities.[1] Herbal plants have been utilized for thousands of years to treat a variety of ailments across the world. According to the World Health Organization (WHO), 80% of the world's population continues to rely largely on traditional medicines for basic treatment[2]. Herbal medications are more popular than allopathic therapies because they are less expensive, have less side effects, are as effective as conventional pharmaceuticals, are more readily available, and are ecologically benign.[2]

Indonesia has the world's sixth most diverse flora, with 20,000 species, 40% of which are indigenous to the country.[3] The existence of native Indonesian flora is fascinating to research, particularly in the health industry. Furthermore, research on medicinal plants has increased

dramatically in recent years, suggesting a growing interest in natural-based traditional medicine.[4] Indonesia is well-known for its sustainable use of traditional medicine[5]. Traditional Indonesian herbal medicine has been used for hundreds of years in Indonesian society and is still widely used to maintain health and cure ailments due to its perceived safety over chemical pharmaceuticals.[6,7] These herbs have historically played a significant role in Indonesian culture and medicine.[8–10] Herbal plants, such as ginger, turmeric, and betel leaves, have been employed in traditional "Jamu" (herbal medicine) for thousands of years, demonstrating their long-term relevance in sustaining health and well-being. In addition to Jamu, the terminology for herbal medicinal beverages in Indonesia differ widely by location or ethnicity.[11,12]

Bali Province has a long history of treating various diseases with herbal plant components in various forms such as powder, decoction, paste, and oil contained in the Balinese traditional medicine system known as "*Usadha*". The formulation of these medicines is based on two principles, namely utilizing one plant or using many herbs. Combining many herbs, often known as "Polyherbal", is hypothesized to increase therapeutic efficacy because various bioactive chemicals work on various therapeutic targets to accelerate the healing process.[13] Polyherbal has been used for thousands of years in many countries to treat various types of disorders such as diabetes, as an antioxidant, hepatoprotective, anti-inflammatory, and anxiety disorders.

Polyherbal derived from a mixture of *Murraya koenigii* L. Spreng leaves, *Allium sativum* L., *Garcinia quaesita* Pierre fruits, and *Piper nigrum* L. seeds is a common herbal remedy in Sri Lanka for treating diabetes mellitus and dyslipidemia.[14] It has been found to be non-toxic and safe for long-term use. The polyherbal, which includes plant extracts including *Boerhavia diffusa, Solidago virgaurea, Vitex negundo*, and thymoquinone compounds, helps reduce hepatorenal damage caused by CCl_4 through antioxidant and anti-inflammatory processes.[15] AYUSH-64 is an Ayurvedic herbal polyphenol formulation consisting of many herbal components such as *Alstonia Scholaris, Picrorhiza kurroa, Swertia chirata,* and *Caesalpinia crista*, has been used for various disorders including malaria, chronic fever, and joint pain.[16] Herbal mixture consisting of *Cassia absus, Gymnema sylvestre, Nigella sativa,* and *Piper nigrum* has high potential in treating diabetic complications, including liver and kidney damage, dyslipidemia, and oxidative stress.[17] Arthralgex Ayurvedic Polyherbal consisting of 18 plant species did not cause serious side effects on biochemical, hematological, or organ parameters of rats during and after the treatment period.[18] One of the polyherbal of usada formulas is made from a mixture of lime leaves *(Citrus amblycarpa),* 11 white peppercorns *(Piper nigrum* L.), and vinegar. These ingredients are ground until smooth, then mixed and used by drinking. This formula is used to treat paresthesia. Pepper (*Piper nigrum* L), has been known to have many active compounds, two of which have a high abundance of Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-; and Caryophyllene.[19]

The toxicity of herbal medicines has raised widespread concerns, and various side effects (including allergic responses, hepatotoxicity, nephrotoxicity, and cardiac toxicity) have been described in recent years.[20] Plants with medicinal properties should have minimal toxicity due to their long-term use in humans.[21] However, many therapeutic plants used in traditional medicine have been associated with harmful consequences. Conducting toxicity testing in appropriate animal models is essential to ensure the safety of treatments and is at the heart of toxicology. According to OECD standards, acute toxicity studies are an advanced approach to administer single and multiple doses of chemicals and report the primary safety profile of a particular compound.[22]

There are several articles that discuss in an exploratory manner the types of medicinal plants collected in *Usadha* in the province of Bali [23–25]; but no empirical facts have been found regarding the safety or benefits of polyherbal *Usadha*. On the other hand, there is a high demand for polyherbals in health services. Therefore, this study was designed to evaluate the acute toxicity properties of polyherbal *Usadha* formulations on kidney and liver function in Wistar rats. The results of this study will be an excellent effort to encourage manufacturers and researchers to examine the optimal composition of raw materials when designing herbal medicines.[26–28]

MATERIALS AND METHODS

Choice of plant combinations

Lime leaves (*Citrus amblycarpa*) and white pepper (*Piper nigrum* L.) were obtained from local farmers in Bali Province. Both materials were selected for research purposes based on their ethnopharmacological properties and their claims in the Lontar Usadha. Lime leaves and black pepper seeds were separated and dried in the shade. The dried leaves and seeds were then ground and sieved and filtered with a 600 mesh sieve size. The powdered plant samples were stored in sterile airtight zip lock bags at 4 ! until used.[28]

Preparation of crude extract

Each of the simplicia was then extracted by maceration method using 96% ethanol solvent for 48 hours, and re-macerated twice, filtered using filter paper. The filtrate was then concentrated in a vacuum rotary evaporator at a temperature of 45! until a thick extract was obtained and then dried by the freeze-dry method. Usada poliherbal is made from a mixture of three ingredients, namely; white pepper seed extract, lime leaf extract, and vinegar in equal proportions.

Animals

A total of 24 male Wistar rats *(Rattus norvegicus)*, aged three months, weighing 200 g to 250 g were used in this study. The rats were grouped into six groups of four each and then acclimatized for one week to adjust to the temperature and humidity of the research room. The rats were kept in cages containing four rats each, given food and drink as much as they wanted. The rats were given polyherbal usada at a dose according to the treatment group for four weeks through the stomach.

Sub-acute toxicity test

The experiment was conducted following OECD guidelines number 407 in the study.[29] The study used a Completely Randomized Design with six treatments. The treatment was the administration of polyherbal usadha with six dose levels, namely; 0 mg/kgbb/day (E0), 100 mg/kgbb/day (E1), 200 mg/kgbb/day (E2), 300 mg/kgbb/day (E3), 400 mg/kgbb/day (E4), and 500 mg/kgbb/day (E5). Each treatment was repeated four times so that there were 24 research units. The treatment was given for four weeks. Twenty-four hours after the last treatment, the mice were euthanized using ketamine, then blood was taken from the orbital sinus cantus using a capillary pipette and stored in a tube.

Blood biochemical analysis

Biochemical studies were conducted to evaluate the safety profile of the polyherbal extract of usadha. Biochemical investigations included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Blood urea nitrogen (BUN), SC, and Total protein (TP). Blood biochemical parameters were determined using a medical analyzer with fuitest whose working procedure was in accordance with the company's instructions.[1]

Hematological analysis

Blood samples from the treatment and control groups were taken and placed into 3 mL vaculab tubes containing EDTA. The parameters used to indicate hematological conditions were RBC (Red Blood Cell), HGB (Hemoglobin), HCT (Hematocrit), MCV (mean cell volume), MCH (Mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), RDW (Red cell distribution width); WBC (white blood cells), Eo (eosinophils), Baso (basophils) Lymp (Lymphocyte), Neut (Neutrophils), Mono (Monocytes), PTL (Platelets), PDW (Platelet distribution width), MPV (Mean platelet volume), PCT (Plateletcrit). These parameters were calculated using the Sysmex pocH-100i™ Haematology Analyzer and the CBC line kit**.**[1]

Statistical analysis

The measurement data were then tabulated in Microsoft Excel and evaluated statistically using One Way ANOVA with Duncan's Test. Results are presented as mean ± SD. The statistical significance value between groups was at pd"0.05.[29]

RESULTS

Blood Biochemical Levels

The results showed that the administration of Polyherbal *Usadha* had a significant effect (pd"0.05) on ALT and ALP, but not significantly (pe"0.05) on AST, BUN, SC, and TP. The average ALT, AST, BUN, TP, and SC tended to decrease after administering Polyherbal *Usadha*, while ALP increased after a 300 mg/kg bw dose. The mean ALT appeared to be significantly lower than the control after administration of 100 mg/kg bw to

500 mg/kg bw doses. Meanwhile, ALP appeared to be significantly higher than the control at a dose of 400 mg/kg bw (Table 1).

The results showed that ALP levels showed a pattern that varied depending on the dose. The administration of polyherbal *usadha* at doses of 100 and 200 mg/kg bw resulted in lower ALP than the control. Still, it then increased significantly compared to the control at the dose of 400 mg/kg bw (Table 1). This condition indicates that administering polyherbal *usadha* at a dose of 400 mg/kg bw causes a certain metabolic response. The results showed that SC, BUN, and TP were not significantly different from the control (Table 1).

Blood Hematology Levels

Research shows that several hematological parameters after administration of Polyherbal *Usadha* show a pattern of changes that vary and depend on the dose. The administration of Polyherbal *Usadha* had a significant effect (pd"0.05) on MCV, PDW, Basophil, and MPV, but not significantly (pe"0.05) on WBC, Lymphocytes, Monocytes, Eosinophils, RBC, HGB HCT MCH, MCHC, RDW, Neutrophils, PLT. The administration of polyherbal usada at doses of 400 and 500 mg/kg bw resulted in a significantly higher MCV value (pd"0.05) compared to the control, while the doses 100; 200, and 300mg/kg bw resulted in a significant lower PDW (pd"0.05) compared to the control. Dose 300; 400, and 500mg/kg bw resulted in significantly lower basophils (pd"0.05). compared to controls, and doses of 100, and 200mg/kg bw also resulted in significantly lower MPV ($p<0.05$) (Table 2).

DISCUSSION

The administration of polyherbal *usadha* resulted in an average lower ALT, and AST than the control, and even significantly lower ALT than the control. This condition is due to the influence of the active ingredients contained in polyherbal *usadha*, namely pepper and lime. The active compounds of pepper (*Piper nigrum* L) are mostly in the form of alkaloid compounds, including Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)-; and Caryophyllene.[19] Meanwhile, lime leaves *(Citrus amblycarpa)* have also been known to have many active compounds, most of which are essential oils, including Citronellol; Caryophyllene; Hexadecanoic acid, ethyl ester; 1-heptatriacotanol; Phytol; Ethyl 9,12,15-octadecatrienoate; Methyl glycolate,3TMS derivative; 3,7-dimethyl oct-6-en-1-yl stearate; Methyl iso-allocholate; Rhodopine; and Tricyclo [20.8.0.0(7,16) triacontane,1(22),7(16)-diepoxy-.[30] Caryophyllene has significant pharmacological benefits, including antioxidant, anticancer, cardioprotective, anti-inflammatory, hepatoprotective, nephroprotective, gastroprotective, antimicrobial, and immunomodulatory properties.[31] These active ingredients have a good effect on liver and kidney function so that polyherbal *usadha* does not cause hepatocyte damage.

Hepatotoxicity is a side effect that often occurs in treatment. This happens because the liver functions as a center for the disposition of drug metabolism and foreign substances in the body.

Table 1. Blood Biochemical Levels of Wistar Rats After Administration of Polyherbal *Usadha* (Mean ±SE mean)

Dose (mg/ kg bw)	Parameter AST(U/L)	ALT(U/L)	ALP(U/L)	BUN (mg/dl)	SC (mg/dl)	TP (mg/dl)
0	160.75±27.55	85.75±9.73	300.50±62.98	36.95±9.05	0.95±0.02	5.25±0.02
100	150.00±23.98	66.25±5.91 *	215.00±29.90	34.30±5.41	0.63±0.11	5.13±0.21
200	97.75±7.28	61.25±5.68 *	266.25±46.38	32.05±5.93	0.88±0.06	4.85±0.18
300	98.40±7.98	61.72±3.84 *	378.88±31.40	27.29±3.04	0.82±0.07	5.03±0.16
400	107.82±12.06	64.08±2.11 *	460.90±36.40 *	32.53±6.11	0.92±0.01	4.90±0.10
500	108.33±9.63	54.95±0.81 *	341.75±22.31	21.79±3.45	0.85±0.06	5.01±0.13

*Significant with control at 0.05 level. ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase), SC (Serum creatinine), BUN (Blood Urea Nitrogen), TP (Total protein (TP)

Table 2. Blood Hematology Levels of Wistar Rats After Polyherbal *Usadha* Supplementation (Mean ±SE mean)

Parameter	Dose mg/kg bw						Normal values
	Control	100	200	300	400	500	
WBC (10^3/uL)	11.80 ± 1.31	12.60 ± 1.6	12.13 ± 0.74	10.99 ± 0.82	10.56 ± 1.00	10.80 ± 0.85	4.80 – 17.50
Neut (10^3/uL)	2.60 ± 0.25	2.94 ± 0.47	3.28 ± 0.76	4.08 ± 0.69	3.80 ± 1.05	3.35 ± 0.71	1.50 – 7.00
Lymph (10^3/uL) >	7.51 ± 1.01	8.04 ± 1.31	7.37 ± 0.58	5.61 ± 0.23	5.42 ± 0.80	6.13 ± 0.20	1.00 – 3.70
Mono (10^3/uL)	0.81 ± 0.10	0.93 ± 0.17	0.85 ± 0.06	1.11 ± 0.04	1.08 ± 0.14	0.87 ± 0.04	0.00 – 0.70
Eo (10^3/uL)	0.34 ± 0.13	0.42 ± 0.08	0.42 ± 0.13	0.22 ± 0.04	0.16 ± 0.07	0.34 ± 0.08	0.00 – 0.40
Baso (10^3/uL)	0.55 ± 0.12	0.27 ± 0.16	0.22 ± 0.21	0.00 ± 0.00 *	0.00 ± 0.00 *	0.11 ± 0.06*	0.00 – 0.10
RBC (10^6/uL) >	7.65 ± 0.19	7.72 ± 0.17	7.72 ± 0.09	7.35 ± 0.24	6.93 ± 0.27	7.56 ± 0.21	3.90 – 6.10
HGB (g/dl)	13.45 ± 0.23	13.65 ± 0.50	13.45 ± 0.27	13.09 ± 0.42	12.50 ± 0.54	13.70 ± 0.31	11.1 – 18.0
HCT (%)	40.25 ± 0.79	41.38 ± 1.00	41.03 ± 0.32	38.85 ± 0.89	38.12 ± 1.45	41.74 ± 0.74	31.0 – 52.0
MCV (fL) <	52.68 ± 0.60	53.60 ± 0.40	53.13 ± 0.31	52.87 ± 0.60	54.99 ± 0.36 *	55.24 ± 0.68 *	86.0 – 110.0
MCH (pg) <	17.63 ± 0.20	17.65 ± 0.31	17.42 ± 0.30	17.79 ± 0.18	18.02 ± 0.17	18.13 ± 0.21	26.0 – 38.0
MCHC (g/dl)	33.43 ± 0.20	32.95 ± 0.41	32.78 ± 0.50	33.63 ± 0.34	32.77 ± 0.39	32.86 ± 0.25	31.0 – 37.0
RDW_SD (fL)	33.93 ± 2.40	28.95 ± 0.10	29.83 ± 0.49	32.56 ± 1.42	34.31 ± 3.13	32.41 ± 1.46	-
RDW_CV (%)	20.85 ± 0.94	18.25 ± 0.25	18.90 ± 0.09	20.09 ± 0.42	19.86 ± 0.88	19.28 ± 0.28	11.0 – 16.0
PLT (10^6/uL) >	701.25 ± 170.42	946.25 ± 60.98	956.00 ± 168.27	1066.08 ± 68.13	1201.58 ± 234.00	1077.33 ± 118,77	150 – 450
MPV (fL) <	7.80 ± 0.13	7.35 ± 0.12 *	7.38 ± 0.13 *	7.46 ± 0.08	7.55 ± 0.18	7.90 ± 0.13	9.0 – 13.0
PCT (%)	0.54 ± 0.13	0.70 ± 0.03	0.70 ± 0.11	0.80 ± 0.04	0.90 ± 0.17	0.85 ± 0.10	0.17 – 0.35
PDW (fL)	9.20 ± 0.11	8.35 ± 0.25 *	8.35 ± 0.15 *	8.61 ± 0.11 *	8.91 ± 0.26	9.23 ± 0.15	9.0 – 17.0
P_LCR (%)	11.23 ± 1.49	7.23 ± 0.40	7.48 ± 0.77	7.56 ± 0.74	8.50 ± 1.35	10.26 ± 0.81	-

* Significant with control at 0.05 level; > exceeding normal value, < less than normal value. Note: RBC (Red Blood Cell), HGB (Hemoglobin), HCT (Hematocrit), MCV (mean cell volume), MCH (Mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), RDW (Red cell distribution width); WBC (white blood cells), Eo (eosinophils), Baso (basophils) Lymp (Lymphocyte), Neut (Neutrophils), Mono (Monocytes); PTL (Platelets), PDW (Platelet distribution width), MPV (Mean platelet volume), PCT (Plateletcrit).

Drugs given orally to pass through the intestinal cell membrane must be fat-soluble, then taken to the liver to be converted into water-soluble (more polar), then excreted in urine (if the molecule is small), or through bile (if the molecule is large). Liver lesions can occur due to hepatocellular reactions through the production of enzyme-drug complexes. This complex will then cause cell dysfunction, membrane dysfunction, and, cytotoxic response of T cells.[32] A similar study using a mixture of *Asparagus racemosus*, *Cajanus cajan, Cassia fistula,* and *Carissa spinarum*, was able to protect the liver from damage induced by carbon tetrachloride (CCl_4).[33] Other studies also found that *Piper nigrum* stems provided protection against hepatotoxicity and dyslipidemia in tenofovir/lamivudine/efavirenz-induced wistar rats.[34]

The roles of ALT and AST as markers of hepatocellular damage.[34-36] Increased levels of ALT and AST in the bloodstream are generally considered a sign of liver tissue damage [37]; the higher the level in the blood, the higher the liver damage that occurs [38]. ALT and AST are cytosolic enzymes involved in gluconeogenesis. In this process, glucose is synthesized from the amino acids alanine and aspartate and then exported to the blood circulation.[39] AST is found primarily in the mitochondria and cytosol of hepatocytes. Still, it can also be found in the heart muscle and skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes. Nevertheless, ALT is more specific to the liver, being found in much higher concentrations compared to other tissues. Thus, it can be said that the administration of polyherbal *usadha* does not cause damage to hepatocytes.

An increase in ALP, while liver test results are normal, may indicate increased osteoblastic activity or tissue regeneration, but requires additional testing for certainty. ALP enzymes are found in the liver, bones, and placenta. ALP is an enzyme that plays a role in accelerating the hydrolysis of organic phosphate by releasing inorganic phosphates. ALP enzyme runoff in the blood can be used as a marker of cell damage.[40],[41] Lime and pepper mint can protect the kidneys so that they do not cause problems with kidney function. Caryophyllene with high concentrations in polyherbal [19],[30]; is known to have significant pharmacological benefits, including antioxidant, anticancer, cardioprotective, anti-inflammatory, hepatoprotective, nephroprotective, gastroprotective, antimicrobial, and immunomodulatory properties.[31]

The presence of SC, BUN, and TP in the blood is an important indicator of kidney dysfunction. Creatinine is the end product of creatine metabolism excreted through the kidneys.[42] Meanwhile, increased BUN indicates kidney failure in carrying out its filtration function. Meanwhile, total protein plays a role in determining the diseases that cause protein production disorders. Thus, it can be said that the administration of polyherbal *usadha* does not cause changes in the liver and kidney function of Wistar rats, so it is relatively safe when used as a medicinal ingredient up to a certain dose.

The results showed that the RBC value at all doses was higher than the normal value. This suggests the potential effects of polyherbal *usadha* supplementation on red blood cell production but does not significantly affect hemoglobin concentrations. This suggests that an increase in RBC is not accompanied by changes in red blood cell quality or excessive oxygenation capacity. Other erythrocyte parameters (MCV, MCH) were generally below normal values at low doses, but MCV showed significant differences compared to controls after polyherbal supplementation at doses of 400 and 500mg/kg bw. A slightly lower MCV value after low-dose polyherbal supplementation could indicate that at low doses, this supplement has the potential to lower red blood cell size, but this effect does not lead to anemia or iron deficiency, given that MCH and HGB remain stable.[43]

Red blood cell production is a complex and multi-layered process that is primarily regulated by erythropoietin (Epo). The main physiological regulator of EPO production is renal hypoxia or hypoxemia which both reduce the oxygen-carrying capacity of the blood. EPO gene expression is controlled by a transcription factor known as hypoxia-inducible factor (HIF) which plays an important role in cellular adaptation to decreased oxygen levels. Once released from the kidneys, the EPO circulates to the hematopoietic marrow which binds to and activates its receptors (EPOR). EPOR in turn phosphorylates and activates the signal transduction of Janus kinase-2 (JAK-2), and Signal Transducer and Activator of

Transcription-5 (STAT-5) in erythroid progenitors, as well as phosphatidylinositol-3 (PI-3) kinase. Signals transduced through JAK-2/STAT-5 and PI-3 kinase cause changes in gene expression that promote effective erythropoiesis by increasing proliferation and reducing red blood cell progenitor apoptosis.[44]

The mean WBC count remained within the normal range (4.80–17.50 10^3/uL) at all doses, with no significant changes indicating serious adverse effects on the immune system. The number of neutrophils increases with doses up to 300 mg/kg but is still within normal limits. In contrast, lymphocytes show higher values than normal, but show a decrease at higher doses, especially at 300-500 mg/kg. An increase in neutrophils indicates a mild immune response, while a decrease in lymphocytes at high doses signals an adjustment of the immune system to polyherbal components without any indication of serious immune damage or suppression.[45]

Platelet counts at all doses of supplementation, and especially at high doses reaching 1201.58 10v /uL, well above the normal range (150–450 10v /uL). This response may be caused by polyherbal components that promote platelet formation. MPV and PDW tended to decrease at low doses compared to the control group, suggesting specific effects on platelet size and distribution at low doses of this supplement. At low doses, it suggests that polyherbal may affect the average size of platelets, but this is not accompanied by changes that compromise platelet function. This condition is likely the result of infection, chronic inflammation, or drug reactions, which are the most common causes of thrombocytosis.[46,47]

Overall, polyherbal *usadha* supplementation exerted a significant influence on several hematological parameters in Wistar rats, specifically on the increase in platelets and RBCs without any visible hematological side effects. This supplementation appears to be able to support the hematopoiesis process, which is potentially beneficial in certain conditions that require an increase in platelet count or erythrocytes. However, given the fairly high increase in platelets, more research is needed to monitor the potential risk of hypercoagulability or increased blood clots. This study provides a solid basis for further studies regarding the benefits and risks of using polyherbal *usadha* in clinical applications.

CONCLUSION

Polyherbal usadha supplementation in Wistar rats affects several hematological and biochemical parameters. The main effects appear to be immune system stimulation and platelet formation. In addition, the positive impact of ALT shows potential benefits in supporting liver function. However, the increase in ALP at high doses should be looked at further, as it could indicate the potential for liver stress at higher doses. Polyherbal usadha appears consumable without the risk of significant side effects on blood parameters and liver function. Additional studies that include histological analysis and mechanisms of action will provide deeper insights into the therapeutic effects of Polyherbal usadha.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The use of experimental animals in this study has received ethical approval from the animal ethics committee of the Faculty of Veterinary Medicine, Udayana University (UNUD) with Number: B/110/UN14.2.9/PT.01.04/2024.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

I Nyoman Arsana: Conceptualization, Methodology, Data collection, Data analysis, Project administration, and Funding Acquisition; Ni Ketut Ayu Juliasih and Anak Agung Ayu Sauca Sunia Widyantari: Data collection, Data analysis, and Project administration; I Gede Widhiantara and Putu Angga Wiradana: Writing – Original Draft, Editing, Supervision, and Visualization.

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