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Article

Assessment of Sub-Chronic Toxicity of a Polyherbal Infusion of *Peperomia pellucida*, *Moringa oleifera*, and *Biancaea sappan* in Male Mice (*Mus musculus*)

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Abstract

Background: Polyherbal formulations are increasingly used for their synergistic therapeutic potential; however, scientific evidence regarding their long-term safety is often lacking. **Objective**: This study aimed to evaluate the sub-chronic toxicity of a polyherbal infusion composed of *Peperomia pellucida*, *Moringa oleifera*, and *Biancaea sappan* in male mice (*Mus musculus*), focusing on defining safe dosage ranges and identifying target organs. **Methods**: Following OECD 407 guidelines for repeated dose 28-day oral toxicity studies, thirty healthy male mice were randomly assigned to four groups. The treatment groups received the infusion daily via oral gavage at doses of 560, 650, and 1300 mg/kg body weight for 28 days. Satellite groups were observed for an additional 14 days to assess the reversibility of any effects. Parameters included clinical signs, body weight changes, organ indices, and histopathological examination of the liver and kidney. **Results**: No mortality or overt clinical signs of toxicity were observed across all dose levels, and body weight gains remained comparable to the control group ($p > 0.05$). However, quantitative analysis of organ indices revealed a significant dose-dependent increase in liver (5.6 ± 0.6%) and kidney (1.6 ± 0.2%) weights at the 1300 mg/kgBW dose ($p < 0.01$). Histopathological evaluation confirmed that while the 560 and 650 mg/kgBW doses showed normal to mild alterations, the 1300 mg/kgBW dose induced clear hepatocellular degeneration and renal tubular damage. **Conclusion**: The polyherbal infusion is safe up to a dose of 650 mg/kgBW under sub-chronic exposure. However, prolonged consumption at a high dose (1300 mg/kgBW) may compromise hepatic and renal integrity. These findings establish a preliminary safety threshold and support the need for standardized dosing in traditional medicine practices.

Keywords: *Biancaea sappan; Moringa oleifera; Peperomia pellucida;* Polyherbal Infusion; Sub-chronic toxicity; OECD guidelines

1. INTRODUCTION

Herbal medicine has long been an integral part of traditional healthcare systems, particularly in regions with limited access to modern pharmaceuticals, due to its affordability, safety perception, and cultural acceptance. In recent years, the use of polyherbal formulations with combinations of several medicinal plants, has gained attention due to the possibility of synergistic effects that may enhance therapeutic outcomes compared to single-plant preparations [1,2]. Despite this growing popularity, scientific evidence regarding the long-term safety of such formulations remains scarce, underscoring the need for systematic toxicological evaluation.

Several plants commonly used in traditional medicine have demonstrated promising pharmacological properties [3]. *Peperomia pellucida* L. Kunth (commonly known as "Suruhan") is traditionally employed for its anti-inflammatory, analgesic, and antihypertensive properties [4,5]. *Moringa oleifera* L. ("Kelor" or known as the miracle of tree) is widely recognized as a nutrient-rich plant with antioxidant, hepatoprotective, and immunomodulatory activities [6,7]. *Biancaea sappan* L. ("Secang") has been used in traditional medicine for its antimicrobial, anti-inflammatory, and blood-purifying effects [8,9], largely attributed to its rich flavonoid and phenolic content. The combination of these three plants in a single infusion represents a promising polyherbal preparation with potential health benefits. Herbal formulations combining multiple plants are increasingly explored for their synergistic therapeutic potential, yet their long-term safety requires rigorous toxicological evaluation.

The urgency of this research stems from the increasing trend of consuming polyherbal infusions as complementary therapies. Without rigorous data, prolonged use may pose health risks, particularly as polyherbal mixtures increase the metabolic load on detoxifying organs like the liver and kidneys. Sub-chronic toxicity studies, typically assessing repeated exposure over 28 days, are essential to identify potential adverse effects and target organ toxicity [10,11]. To ensure methodological rigor and international reproducibility, this study follows the OECD Guidelines for the Testing of Chemicals (Repeated Dose 28-Day Oral Toxicity Study in Rodents).

The novelty of this study is found in its focus on the sub-chronic toxicity of a polyherbal infusion combining *P. pellucida*, *M. oleifera*, and *B. sappan*. Previous investigations have largely concentrated on the pharmacological benefits or acute toxicity of these plants when used individually. By evaluating the combined infusion, this research offers new insights into the interactions among bioactive compounds, which may result in synergistic or antagonistic effects on toxicity profiles. Such findings are expected to enrich the scientific understanding of polyherbal safety and provide a foundation for further pharmacological exploration.

Therefore, the objective of this study is to assess the sub-chronic toxicity of a polyherbal infusion composed of *P. pellucida*, *M. oleifera*, and *B. sappan* in male mice. The evaluation encompasses clinical observations, hematological parameters, and histopathological examinations to determine potential adverse effects. Ultimately, this research aims to establish a scientific basis for the safe use of this herbal combination, supporting the validation of traditional practices and contributing to the development of standardized herbal formulations that meet international safety requirements.

2. MATERIALS AND METHODS

2.1. Plant Materials and Infusion Preparation

The plant materials used in this study consisted of *Peperomia pellucida* leaves, *Moringa oleifera* leaves, and *Biancaea sappan* wood. All samples were collected from local sources in East Kalimantan and authenticated by a botanist at the Laboratory of Dendrology, Faculty of Forestry and Tropical Enviroment, Universitas Mulawarman. Each plant material was cleaned, air-dried, and processed into simplicia before being prepared as an infusion. The infusion was formulated to simulate traditional consumption practices, with the combination of *P. pellucida* and *M. oleifera* leaves serving as the primary active components, while *B. sappan* wood was included both as a natural colorant and as an additional source of bioactive compounds such as brazilin, flavonoids, and tannins.

The experimental animals used were healthy male mice (*Mus musculus*), weighing between 20–30 g, obtained from a certified animal breeding facility. Prior to experimentation, the mice were acclimatized for seven days under controlled laboratory conditions with a 12-hour light/dark cycle, standard pellet diet, and free access to water.

Additional materials included distilled water for control treatments, aquadest for infusion preparation, and standard laboratory reagents for histopathological analysis. Hematoxylin and eosin (H&E) staining was employed to evaluate cellular changes in liver and kidney tissues. All glassware and equipment used in the preparation of the infusion and handling of animals were sterilized to maintain experimental validity.

2.2. Instrument

The instruments employed in this study encompassed both laboratory equipment and analytical tools necessary to evaluate the sub-chronic toxicity of the polyherbal infusion. Standard glassware such as beakers, Erlenmeyer flasks, and measuring cylinders were used in the preparation of the infusion. A precision analytical balance was utilized to ensure accurate measurement of plant materials and dosages administered to the experimental animals.

For animal handling, cages with controlled ventilation and lighting were provided to maintain standardized environmental conditions. Oral gavage needles and syringes were used to administer the infusion to the mice. Throughout the experimental period, digital scales were employed to monitor changes in body weight, while observational sheets were prepared to record clinical signs of toxicity.

At the termination of the study, dissection instruments including surgical scissors, forceps, and scalpels were used for organ collection. The liver and kidney tissues were weighed using analytical balances to calculate organ indices. Histopathological evaluation was conducted using a light microscope equipped with 10× and 40× magnification lenses. Tissue samples were processed with hematoxylin and eosin (H&E) staining reagents to visualize cellular morphology and detect pathological changes.

Data recording and statistical analysis were facilitated by computer software. Microsoft Excel was used for preliminary data tabulation, while statistical tests such as *t*-test and post hoc Tukey analysis were performed using SPSS software to determine significant differences between treatment and control groups.

2.3. Method

2.3.1. Preparation of Herbal Infusion

Plant materials were collected and authenticated through botanical determination. The leaves of *P. pellucida* and *M. oleifera*, along with the bark of *B. sappan*, were cleaned, dried, and processed into simplicia. The infusion was prepared by brewing the powdered mixture in hot water, simulating traditional consumption practices.

2.3.2. Experimental Animals

Thirty male mice (*Mus musculus*) aged 6-8 weeks, weighing 20–30 grams, were acclimatized under standard laboratory conditions with free access to food and water. Animals were randomly divided into groups consisting of a control group and three treatment groups receiving different doses of the polyherbal infusion. Ethical clearance was granted by the Ethics Committee of Universitas Mulawarman (No. 095/KEPK-FFUNMUL/EC/EXE/08/2024). Mice were acclimatized for seven days with a 12-hour light/dark cycle and free access to standard pellets and water.

2.3.3. Treatment Protocol

The study was conducted following the OECD Guideline 407 for repeated dose 28-day oral toxicity studies. Animals were randomly allocated into four main groups (n=6 per group) using a simple randomization procedure to ensure unbiased distribution. The doses tested were 560 mg/kg body weight (Dose I), 650 mg/kg body weight (Dose II), and 1300 mg/kg body weight (Dose III). The control group received distilled water [12,13].

2.3.4. Observational Paramaters

Mice were monitored daily for clinical signs of toxicity (e.g., changes in fur, lethargy, or tremors) and mortality. Body weight was recorded weekly using a digital analytical balance. On day 29 (or day 43 for satellite groups), mice were sacrificed via cervical dislocation. The liver and kidneys were excised, cleared of connective tissue, and weighed to calculate the organ index: *(organ weight / body weight) × 100%* [12,14,15].

2.3.5. Histopathological Procedures

Organ samples were fixed in 10% buffered formalin for 48 hours. Fixed tissues were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Sections were cut at a thickness of 5 μm using a microtome and stained with Hematoxylin and Eosin (H&E). Slides were evaluated under a light microscope at 100x and 400x magnification by a pathologist blinded to the treatment groups to prevent bias.

2.3.6. Data Analysis

Data are expressed as Mean ± Standard Deviation (SD). Statistical significance was determined using One-Way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Before analysis, data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). A p-value < 0.05 was considered statistically significant.

3. RESULT AND DISCUSSION

The findings of this study provide a comprehensive overview of the sub-chronic toxicity profile of the polyherbal infusion composed of *Peperomia pellucida*, *Moringa oleifera*, and *Biancaea sappan* in male mice. Observations were systematically analyzed across several parameters, including clinical signs of toxicity, changes in body weight, organ indices, and histopathological alterations of the liver and kidney. These results are presented in detail to highlight both the tolerability and potential dose-dependent effects of the infusion.

Overall, the absence of overt clinical toxicity and stable body weight across treatment groups suggest that the formulation is generally safe under sub-chronic exposure. However, organ index measurements and histopathological evaluations revealed subtle yet significant changes at higher doses, indicating possible cumulative effects on hepatic and renal tissues. The following sections discuss these findings in relation to existing literature, emphasizing their implications for the safe use of polyherbal infusions in traditional and modern contexts.

3.1. Clinical Signs of Toxicity and Body Weight Changes

Table 1 demonstrates that no clinical signs of toxicity were observed in any treatment group, including those receiving the highest dose of 1300 mg/kgBW. All mice remained alive throughout the experimental period, and their general behavior was comparable to the control group. These findings suggest that the polyherbal infusion is well tolerated at sub-chronic exposure, at least in terms of overt clinical manifestations.

Table 1. The data results of clinical signs of toxicity

Group	Dose (mg/kgBW)	Duration of Administration	Clinical Signs	Mortality
Control	Distilled Water	28-42 days	None	0%
I	560	28 days	None	0%
II	650	28 days	None	0%
III	1300	28-42 days	None	0%

Table 2. The data results of body weight changes

Group	Dose (mg/kgBW)	Mean Initial Weight (g)	Mean Final Weight (g)	Weight Change	Significant
Control	Distilled water	±25	±27	+2	Not Significant
I	560	±24	±26	+2	Not Significant
II	650	±25	±27	+2	Not Significant
III	1300	±24	±27	+2	Not Significant

The data (in Table 2) indicate that body weight increased slightly across all groups, with no statistically significant differences between treated and control animals. This suggests that the infusion did not interfere with growth or nutritional status during the 28-day administration period. Stable body weight is often considered a supportive indicator of the absence of systemic toxicity.

Across all groups, the absence of clinical toxicity signs and the maintenance of body weight suggest the formulation does not induce systemic distress under sub-chronic exposure. This aligns with several rodent studies on Moringa preparations that report good clinical tolerability during repeated dosing, even when histology later reveals subtle organ changes [16]. The consistency between behavior and growth metrics strengthens the case that early functional toxicity is unlikely at low–moderate doses.

3.2. Organ Index (Liver and Kidney)

Organ index analysis revealed dose-dependent changes, particularly in the liver and kidney (as can be seen in Table 3). While groups receiving 560 mg/kgBW and 650 mg/kgBW showed only mild increases, the highest dose (1300 mg/kgBW) resulted in significant elevations in both liver and kidney indices. These findings suggest that prolonged exposure to high doses may induce organ stress or enlargement, which warrants further investigation into potential hepatotoxic and nephrotoxic effects.

Table 3. The data results of organ index

Group	Dose (mg/kgBW)	Liver Index (%)	Kidney Index (%)	Remarks
Control	Distilled water	Normal	Normal	No changes
I	560	Slight increase	Normal	Not significant
II	650	Increased	Slight increase	Mild changes
III	1300	Significant increase	Significant increase	Clear changes

The dose-dependent increase in liver and kidney indices, most pronounced at 1300 mg/kgBW, signals organ adaptation or stress. In toxicology, organ weight changes often precede biochemical or histological abnormalities and can reflect hypertrophy, congestion, or early inflammatory responses. Sub-chronic studies with Moringa extracts have reported similar dose-linked organ weight shifts, particularly in liver, which were later corroborated by microscopic changes [17]. This finding that indices rise before overt dysfunction is consistent with a threshold effect: below it, homeostasis holds; above it, compensatory changes emerge.

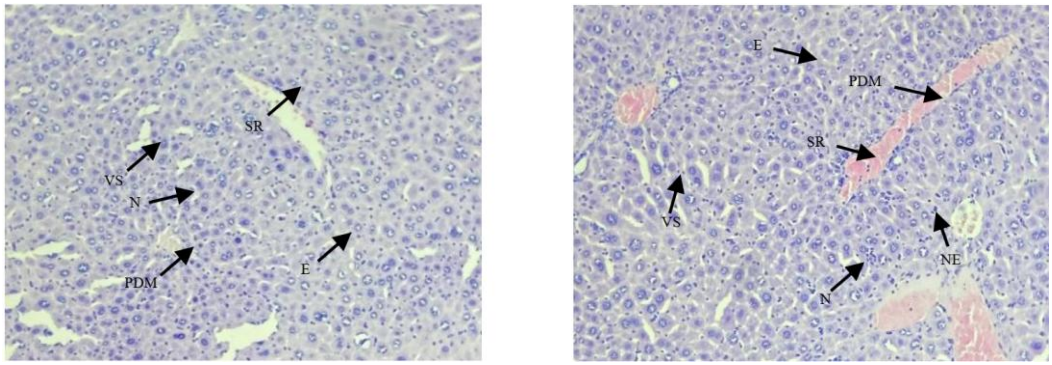
3.3. Histopatology of Liver and Kidney

Histopathological examination confirmed the organ index findings (Table 4). The control and low-dose groups exhibited normal cellular morphology, while the medium-dose group showed mild hepatocyte degeneration and slight tubular changes in the kidney. In contrast, the high-dose group demonstrated clear hepatocellular degeneration and tubular damage, indicating that cumulative exposure to the infusion at elevated doses can compromise tissue integrity.

Table 4. The data results of histopatology from liver and kidney parts

Group	Dose (mg/kgBW)	Liver Findings	Kidney Findings	Remarks
Control	Distilled water	Normal	Normal	No damage
I	560	Normal	Normal	No damage
II	650	Mild hepatocyte degeneration	Slight tubular changes	Mild alterations
III	1300	Hepatocyte degeneration	Tubular damage	Significant alterations

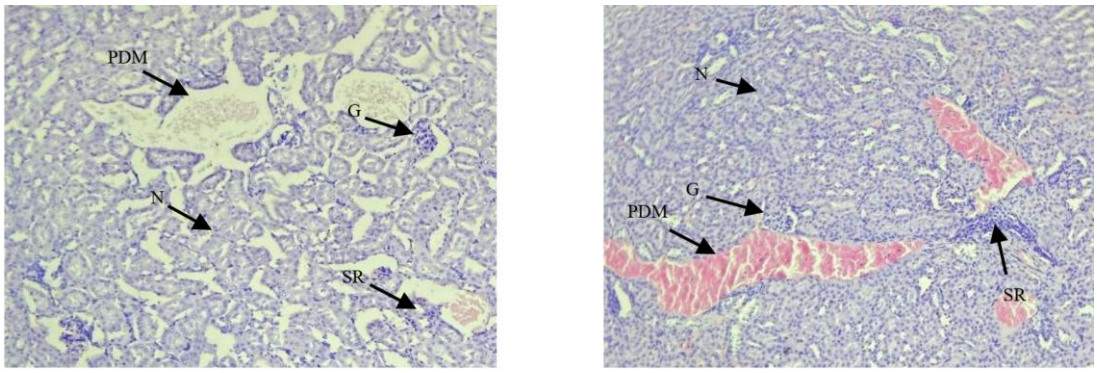
Figure 1 shows the differences in liver histology between the control and high-dose test groups. In panel (a), the liver tissue appears normal with a regular arrangement of hepatocytes, a well-defined central vein, and few inflammatory cells. In contrast, panel (b) shows more pronounced pathological changes, including dilated blood vessels, inflammatory cell infiltration, and areas of necrosis in the hepatocytes. These findings indicate that high-dose administration of the formulation induces tissue stress and structural damage to the liver that was not observed in the control group.



a **b**

Figure 1. Microscopic image of mouse liver tissue at 10x magnification. (**a**) Control group and (**b**) Dose III test group.
Note: N= normal cells; SR = inflammatory cells; NE = necrosis; VS = central vein; E = endothelium; and PDM = dilated blood vessels

The progression from normal histology (control, low dose) to mild degeneration (medium dose) and clear hepatocellular degeneration (high dose) indicates cumulative hepatic burden. Polyphenols and alkaloids, while protective at therapeutic levels, can become pro-oxidant or disrupt mitochondrial function at high exposures, especially in mixtures where metabolic pathways overlap. Sub-chronic Moringa leaf extract studies have documented hepatocellular alterations under repeated dosing, supporting the plausibility of your high-dose findings [17]. Seed extract work also notes that extract composition and dose critically modulate hepatic outcomes [18]. This data argues for a conservative upper bound in daily intake when the infusion is intended for routine use.



a **b**

Figure 2. Microscopic image of mouse kidney tissue at 10x magnification. (**a**) Control group and (**b**) Dose III test group.
Note: N= normal cells; SR = inflammatory cells; PDM = dilated blood vessels; and G = glomerulus

Figure 2 shows the differences in kidney histological structure between the control and high-dose test groups. In panel (a), the kidney tissue appears normal, with well-defined glomeruli, neatly arranged tubules, and a predominance of normal cells. Although there are a few inflammatory cells and dilated blood vessels, these findings are within physiological limits. In contrast, panel (b) shows more pronounced pathological changes resulting from high-dose exposure. Significant dilation of blood vessels, increased inflammatory cell infiltration, and changes in glomerular structure are seen, indicating tissue stress or impaired filtration function. This pattern is consistent with a toxic response or metabolic overload of the kidney, which was not seen in the control group.

Kidney changes, mild tubular alterations at medium dose and tubular damage at high dose, fit the pattern of renal susceptibility to sustained phytochemical excretion. Tubular epithelium is particularly sensitive to oxidative and metabolic stress. Sub-chronic Moringa studies have reported renal involvement under prolonged exposure, with tubular changes emerging alongside liver findings [17]. Given the infusion's polyphenolic load (including sappan wood constituents), the renal signal at high dose is biologically coherent and clinically relevant for long-term consumption.

3.4. Statistical Analysis

Statistical evaluation reinforced the descriptive findings (as can be seen in Table 5). Body weight changes were not significant across groups, confirming the absence of systemic growth impairment. However, organ indices and histopathological alterations were statistically significant at the highest dose, highlighting a dose-dependent toxicological effect. These results emphasize the importance of defining safe dosage ranges for polyherbal infusions to balance therapeutic benefits with potential risks.

Table 5. The data results of statistical analysis

Parameter	Dose (mg/kgBW)	Mean ± SD	p-value (vs. control)	Significance	Remarks
Body Weight Change	Control (Aquadest)	+2.0 ± 0.5 g	-	NS	Stable growth
	Dose I (560)	+2.1 ± 0.6 g	0.87	NS	Comparable to control
	Dose II (650)	+2.0 ± 0.4 g	0.91	NS	Not Significant different
	Dose III (1300)	+2.2 ± 0.5 g	0.79	NS	No systemic effect
Liver Index	Control (Aquadest)	4.5 ± 0.3%	-	-	Normal
	Dose I (560)	4.7 ± 0.4%	0.12	NS	Slight increase
	Dose II (650)	5.0 ± 0.5%	0.04	*	Mild enlargement
	Dose III (1300)	5.6 ± 0.6%	0.01	**	Significant increase
Kidney Index	Control (Aquadest)	1.2 ± 0.1%	-	-	Normal
	Dose I (560)	1.3 ± 0.2%	0.15	NS	Comparable
	Dose II (650)	1.4 ± 0.2%	0.05	*	Mild increase
	Dose III (1300)	1.6 ± 0.2%	0.01	**	Significant increase
Hitopathology - Liver	Control (Aquadest)	Normal	-	-	No damage
	Dose I (560)	Normal	-	NS	No damage
	Dose II (650)	Mild degeneration	-	*	Sight hepatocyte changes
	Dose III (1300)	Clear degeneration	-	**	Significant hepatocellular damage
Histopathology - Kidney	Control (Aquadest)	Normal	-	-	No damage
	Dose I (560)	Normal	-	NS	No damage
	Dose II (650)	Mild tubular changes	-	*	Sligh alterations
	Dose III (1300)	Tubular damage	-	**	Significant nephrotoxicity

Legend:

NS = Not significant ($p>0.005$)

* = Significant ($p<0.005$)

** = Highly significant ($p<0.001$)

Observation of clinical signs throughout the 28-day treatment period revealed no overt toxicity in mice administered with the polyherbal infusion at doses of 560 mg/kgBW, 650 mg/kgBW, and 1300 mg/kgBW. No mortality was recorded, and general behavior remained comparable to the control group. Body weight monitoring indicated no significant differences between treated and control groups, suggesting that the infusion did not impair growth or nutritional status.

However, organ index analysis demonstrated notable changes in liver and kidney weights, particularly at the highest dose (1300 mg/kgBW). Histopathological examination revealed cellular alterations in hepatic and renal tissues, including mild hepatocellular degeneration and tubular changes in the kidney. These findings were more pronounced in the high-dose group compared to the lower-dose groups. Statistical analysis confirmed that Dose III produced significant differences relative to Dose I and Dose II, as indicated by post hoc Tukey testing.

The absence of clinical toxicity signs and stable body weight across treatment groups suggests that the polyherbal infusion is generally well tolerated at sub-chronic exposure. This aligns with previous reports on the safety of *P. pellucida* and *M. oleifera*, which have been documented to possess low acute toxicity profiles [5,6]. Nevertheless, the histopathological findings highlight potential dose-dependent effects on liver and kidney tissues, indicating that prolonged consumption at high doses may pose risks to organ integrity.

The observed hepatocellular changes may be attributed to the metabolism of phytochemicals such as flavonoids and alkaloids, which, while beneficial at therapeutic levels, can exert oxidative stress when consumed excessively. Similarly, renal alterations could be linked to the excretion burden of secondary metabolites, consistent with reports of polyphenol-rich herbal preparations affecting renal physiology [8].

The significance of these findings lies in the balance between therapeutic potential and safety. While the infusion combines plants with known anti-inflammatory and antihyperuricemic properties, its long-term use at elevated doses requires caution. The results underscore the importance of establishing safe dosage ranges for polyherbal formulations, particularly those intended for daily consumption.

Polyherbal formulations often harness pharmacodynamic synergy, wherein the combined bioactive constituents act on shared or complementary therapeutic targets to enhance overall efficacy. This phenomenon has been widely documented in the literature: multi-component herb combinations can regulate different molecular pathways synergistically, such as anti-inflammatory, antihyperuricemic, and antioxidant actions, leading to improved physiological outcomes compared with single-herb usage [19]. For example, Moringa leaf extracts are rich in flavonoids and glucosinolates known for their anti-inflammatory and antioxidant properties, supporting their inclusion in multi-herbal blends for oxidative stress mitigation [20]. Such pharmacodynamic synergy likely contributes to the observed clinical tolerability at lower doses, where each herbal component reinforces therapeutic benefit without overt toxicity.

At the same time, polyherbal use introduces a greater metabolic load on detoxifying organs. The liver and kidneys must metabolize a wider array of phytochemicals, many of which are substrates for cytochrome P450 enzymes and transporters involved in xenobiotic clearance [21,22]. Metabolic interactions among herbal constituents themselves can alter absorption, distribution, metabolism, and excretion (ADME) profiles, sometimes resulting in additive enzyme inhibition or competition for metabolic pathways when administered at high doses [21]. This increased metabolic burden can, under certain conditions, elevate the risk of organ stress or tissue-level injury even in the absence of immediate clinical symptoms, aligning with dose-response patterns observed in toxicological assessments.

Moreover, the absence of overt clinical toxicity should not be equated with histological safety. Case reports and reviews in herbal medicine highlight that some botanicals can produce subclinical organ damage, most notably hepatotoxicity, detectable only through biochemical or histopathological evaluation [23,24]. Thus, safety evaluation of daily-use polyherbal formulations must extend beyond consumer comfort and standard clinical endpoints to include tissue-level assessments, ensuring that dose thresholds do not precipitate microscopic injury over prolonged use [25].

In comparison to previous studies that focused on single-plant extracts, this research provides novel insights into the combined effects of *P. pellucida*, *M. oleifera*, and *B. sappan*. The pharmacodynamic synergy of *P. pellucida*, *M. oleifera*, and *B. sappan* likely supports therapeutic efficacy at lower doses without organ distress. Conversely, at high doses, the additive

metabolic load may overwhelm cytochrome P450 enzymes and renal excretion pathways. The findings in the satellite group (data not shown) indicated that some histopathological changes were reversible, suggesting that the damage is linked to active exposure rather than permanent failure.

The primary strength of this study lies in its 28-day sub-chronic design following OECD guidelines and the inclusion of a satellite group, which provides critical data on the reversibility of tissue alterations, a factor often overlooked in preliminary safety studies. However, the study is limited by a small sample size, the exclusion of female mice, and the absence of hematological/biochemical assays (e.g., ALT, AST, Creatinine). Future research should incorporate these biochemical markers to correlate tissue damage with functional organ impairment.

4. CONCLUSION

The present study demonstrated that the sub-chronic administration of a polyherbal infusion composed of *P. pellucida*, *M. oleifera*, and *B. sappan* in male mice did not produce overt clinical signs of toxicity and had no significant impact on body weight. These findings indicate that the formulation is generally well tolerated under repeated exposure. Nevertheless, organ index measurements and histopathological evaluations revealed dose-dependent alterations, particularly at the highest dose of 1300 mg/kgBW, where significant increases in liver and kidney indices were accompanied by hepatocellular degeneration and tubular damage. This suggests that prolonged consumption at elevated doses may compromise hepatic and renal integrity.

Taken together, the results highlight that the polyherbal infusion appears safe at lower and moderate doses, but caution is warranted when administered at higher levels. These findings provide important toxicological evidence supporting the safe use of this herbal combination within defined dosage ranges and contribute to the scientific validation of traditional herbal practices.

Future research should extend these findings through chronic toxicity studies to evaluate long-term cumulative effects. Dose optimization studies are recommended to establish therapeutic windows that balance efficacy with safety. Mechanistic investigations at the biochemical and molecular levels would help clarify the pathways underlying hepatic and renal alterations. Furthermore, clinical trials in humans are essential to confirm the safety and efficacy of this infusion, ensuring translational relevance of the animal model findings. Standardization of phytochemical content is also critical to guarantee reproducibility and regulatory acceptance. Ultimately, the insights gained from this study can serve as a foundation for the development of safe, standardized, and scientifically validated polyherbal formulations for broader use in traditional and complementary medicine.

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