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# HISTOLOGICAL AND BIOCHEMICAL MARKERS ON THE LIVER OF WISTER ALBINO RATS SUPPLEMENTED WITH FLOWER-HEAD AND LEAF EXTRACTS OF *SPILANTHES FILICAULIS*

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***Abstract***

*This study covered the effects of Spilanthes filicaulis flower-head and leaf extracts on liver function and histology in Wistar albino rats. Forty-two rats were divided into seven groups, with groups receiving varying doses of MESF leaf or flower-head extracts. Liver function was assessed through serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin fractions. Histopathological analysis of liver tissues was performed to detect structural changes. The results indicated that neither the leaf nor flower-head extracts caused significant alterations in serum ALT and AST levels, suggesting no major hepatotoxicity. Although some variations in ALP activity were observed, these changes did not correlate with liver damage in histopathological examinations. Bilirubin levels remained stable across treatment groups. Histopathological analysis revealed no significant deviations from normal hepatic architecture, though some cases of macrovesicular steatosis were noted. The study concludes that supplementation with MESF leaf and flower-head extracts does not induce significant liver damage in Wistar albino rats, suggesting that these extracts may be safe for liver health at the administered doses.*

**Keywords:** *Spilanthes filicaulis*, medicinal plant, hepatoprotective, anti-inflammatory, herbal medicine, Asteraceae

## Introduction

The liver is a central organ in detoxification and metabolic processes, making its health critical for overall well-being. Biochemical and histological markers are essential for assessing liver function and damage (Moatamed et al, 2019). Recent studies have explored the effects of various plant extracts on liver health, with a focus on traditional medicinal plants. One such plant is *Spilanthes filicaulis*, known for its potential therapeutic benefits. *Spilanthes filicaulis* is a medicinal plant native to tropical regions, including parts of Nigeria (Ojo et al, 2024). Known for its therapeutic properties, it belongs to the Asteraceae family. The plant features small, vibrant yellow flower-heads and lance-shaped leaves, which are traditionally used in herbal medicine. Its bioactive compounds are believed to possess anti-inflammatory, analgesic, and hepatoprotective effects. In traditional practices, *Spilanthes filicaulis* is used for its potential benefits in digestive health and liver function, although scientific research on its full pharmacological effects is

still ongoing (Ojo et al, 2023). The plant is valued for its potential in natural health treatments.

Groups of blood tests that offer details about a patient's liver condition are known as liver function tests. According to Lee (2009). Amino acids are synthesized, broken down, and converted into molecules that store energy by liver-resident transaminases. Typically, serum concentrations of these transaminases are negligible. Nevertheless, in the event of hepatocyte injury, the liver cell membrane becomes more permeable, allowing some of the enzymes to escape into the bloodstream. According to Johnston (1999), transaminases that are often tested include aspartate and alanine transaminases. Elevated levels have the potential to cause liver damage, therefore they are probably going to be there if something is wrong. They could also be more common in other illnesses such thyroid issues, muscular problems, and celiac disease (Hustead and Oh, 2011). Severe liver damage, such as viral hepatitis, liver injury from reduced blood flow, or harm from medicines or poisons, is indicated by extremely high elevations of these two transaminases.

Biochemical assays are vital for evaluating liver function and damage. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are key liver enzymes used to diagnose hepatocellular injury. Elevated levels of these transaminases typically indicate liver damage (Ibeh et al, 2013). In a study by Ewhea et al, (2023), the administration of *Spilanthes filicaulis* leaf and flower-head extracts did not lead to significant increases in ALT and AST levels in Wistar albino rats. This finding suggests that the extracts may not cause hepatocellular damage at the doses tested. These results align with the work of Akoachere et al, (2015), who also reported that *Spilanthes filicaulis* did not significantly alter serum transaminase levels, indicating a lack of acute liver injury.

Alkaline phosphatase (ALP) is another important marker, often used to assess cholestasis or other hepatic dysfunctions (Riancho-Zarrabeitia et al, 2016). Yang et al, (2024) observed varying effects on ALP levels in rats treated with *Spilanthes filicaulis* leaf and flower-head extracts. Specifically, a significant increase in ALP activity was noted in some groups receiving lower doses of the leaf extract, while higher doses did not show a proportionate increase. This suggests that the observed changes in ALP might not be directly related to liver damage but could be influenced by factors such as damage to other tissues where ALP is present (Kaplan, 1972). This observation is consistent with the findings of Onyango et al, (2024), who noted that variations in ALP levels could be influenced by extra-hepatic factors.

Particularly regarding the liver's capacity to conjugate and eliminate bilirubin, bilirubin levels offer more information on liver function. Bilirubin, both conjugated and unconjugated, are measured together to determine total bilirubin levels. Extremely

hydrophobic, unconjugated bilirubin is mostly carried attached to albumin that is circulated in the blood. Haeme is a breakdown product of haemoglobin in red blood cells (Harpavat et al, 2015). High levels of free fatty acids and hydrophobic medications at high concentrations can raise unconjugated bilirubin levels. Unconjugated bilirubin must be removed from the blood by the liver, and each time blood passes through the liver in a healthy person, up to 30% of it is absorbed. A number of issues can be indicated by elevated total bilirubin (TBIL), which also causes jaundice. Indirect bilirubin, or excess unconjugated bilirubin, is the issue if direct (conjugated) bilirubin is normal. This excess is found in the liver, upstream of bilirubin conjugation (Martelanc et al., 2014). It is possible to suspect hemolysis, or internal bleeding. When the level of direct bilirubin is high, it indicates that the liver is properly converting bilirubin but is unable to eliminate it. It is advisable to suspect bile duct blockage caused by malignancy, cirrhosis, hepatitis, or gallstones (Johnston, 1999).

Histological examination provides direct evidence of tissue integrity and pathological changes. The liver's histopathology is crucial for assessing the impact of substances on hepatic architecture. The urgency of studying the histological and biochemical markers in the liver of Wistar albino rats supplemented with *Spilanthes filicaulis* extracts is underscored by the growing interest in natural remedies for liver health and the need for scientific validation of traditional practices. Despite the extensive use of *Spilanthes filicaulis* in traditional medicine, there is a notable gap in rigorous scientific studies evaluating its hepatoprotective efficacy and safety. Traditional knowledge suggests that the plant has potential liver benefits (Hassan et al, 2022), yet empirical evidence remains sparse.

Recent studies have highlighted the increasing incidence of liver diseases globally, including those linked to oxidative stress and inflammation (Cichoż-Lach & Michalak, 2014; Chen et al, 2020), which *Spilanthes filicaulis* might address. However, a comprehensive understanding of its effects on liver histology and biochemistry is crucial for confirming its therapeutic potential and ensuring its safety for human use (Sun et al, 2019). Without detailed investigations into how *Spilanthes filicaulis* impacts liver enzyme levels, bilirubin metabolism, and tissue integrity, its use in clinical settings remains uncertain. Thus, this study is vital to bridge the gap between traditional claims and scientific validation, supporting evidence-based application in liver health management.

## 2.0 Materials and Methods

**Plant Materials:** A large quantity (2736 g) of *Spilanthes filicaulis* was collected from Isuofia village. The plant specimen was authenticated by Mr. Alfred Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu state,

Nigeria. The plant's InterCEDD voucher number is InterCEDD/16291. The flower-heads and leaves of the plant were selected differently, dried under room temperature for several days and then pulverized into fine powder.

**Extraction of Crude Plant Extract.**

Pulverized powder of Spilanthes filicaulis leaf and flower-head were respectively subjected to extraction with 80% methanol. The resulting liquid extracts were filtered using Whatman No.1 filter paper and the residues discarded. The methanol extracts was concentrated in water bath at 450 C and stored at 40 C until used.

**Animals:** Adult male Wistar albino rats (150-200g) were sourced from the animal house of the department of Zoology and Environmental Biology, University of Nigeria Nsukka. The rats were allowed to adapt to the environmental conditions for a week and were fed on normal diet and clean drinking water.

**Grouping of Animals:** The animals used in this study were fifty two albino rats of both sexes with an average weight of 150-200g and arranged into seven groups of six animals each. Group 1 (control) received normal saline orally and normal feed orally. The second group was administered with 100mg/Kg bw of S. filicaulis leaf. Group 3 was administered with S. filicaulis leaf at a dose of 200 mg/Kg bw. Group 4 was administered with S. filicaulis leaf at the dosage of 400 mg/Kg bw. Group 5 were given 100 mg/Kg bw of *S. filicaulis* flower-head. Group 6 were given 200 mg/Kg bw of *S. filicaulis* flower-head. Group 7 were given 400 mg/Kg bw of *S. filicaulis* flower-head. The feeding was by intubation (oral feeding with rubber tube).

**Collection of blood and liver sample:** According to the experimental design, three rats from each group were killed on day 15 and 29, and blood was collected for testing of biochemistry. Blood samples were obtained through cardiac puncture using chloroform as an anaesthetic agent into plain sample tubes. The liver functions were determined by the sera obtained from the blood in plain sample bottles. These animals were euthanized using cervical dislocation and the liver tissues were removed and preserved in 10 percent formalin for histopathological examination.

Biochemical assays were conducted to evaluate serum levels of total and conjugated bilirubin, along with the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

**Alanine Aminotransferase (ALT)** ALT levels were quantified using the Reitman and Frankel (1957) method, as described in the Teco Kit. The assay principle involves the reaction: α-Oxoglutarate+L-Alanine→GPT→L-Glutamate+Pyruvate ALT activity was measured by detecting the concentration of pyruvate hydrazone formed when pyruvate reacts with 2,4-dinitrophenylhydrazine.

**Aspartate Aminotransferase (AST)** AST activity was assessed following the Reitman and Frankel (1957) procedure outlined in the Teco Kit. The principle is: α-Oxoglutarate+L-Aspartate→GOT→L-Glutamate+Oxaloacetate AST levels were determined by measuring the concentration of oxaloacetate hydrazone, which forms a complex with 2,4-dinitrophenylhydrazine.

**Alkaline Phosphatase (ALP)** ALP activity was measured according to the Deutsche Gesellschaft für Klinische Chemie (GSCC, 1972) method, using the Randox kit. The reaction principle is: P-Nitrophenylphosphate+H2O→ALP→Phosphate+P-Nitrophenol (a colored chromogen)

**Bilirubin** The concentrations of conjugated and unconjugated bilirubin were analyzed using the Jendrassik and Grof (1938) method with the Randox kit. This method employs:

- **Direct Bilirubin**: Reacts with diazotized sulphanilic acid in an alkaline environment, producing a blue-colored complex.
- **Total Bilirubin**: Measured after caffeine addition, which dissociates albumin-bound bilirubin, allowing it to react with diazotized sulphanilic acid.

**Histopathological Examination**

The liver tissues of Wistar albino rats were subjected to histopathological analysis following the protocol of Drury et al. (1967). Liver samples were fixed in 10% formalin, processed through dehydration, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Microscopic examination was performed by mounting the slides on a photomicroscope, and images were captured at various magnifications to document the findings.

**Statistical Analysis**

Data were presented as Mean ± SD. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20. Analysis of variance (ANOVA) was employed to assess statistical significance, with a significance level set at $p < 0.05$. Results were compared to control groups, and differences were considered statistically significant at $p < 0.05$.

## 3.0 Results

**Serum AST Activity:** As depicted in Figure 1, the administration of MESF leaf and flower-head extracts led to varied effects on serum aspartate aminotransferase (AST) levels across different experimental groups. In the first batch (14 days), AST levels decreased significantly ($p < 0.05$) in groups five and seven, while groups two, three, four, and six showed a non-significant reduction ($p > 0.05$) compared to the control group. Conversely, in the second batch (28 days), most groups exhibited no significant change ($p > 0.05$) in AST levels, with the exception of group three, which experienced a non-significant

increase. Overall, neither the leaf nor the flower-head extracts had a pronounced effect on AST activity.

**Serum ALT Activity:** Figure 2 illustrates the effect of MESF leaf and flower-head extracts on serum alanine aminotransferase (ALT) activity. In the first batch (14 days), ALT levels remained unchanged ($p > 0.05$) across all groups, except for a non-significant increase in group four. Similarly, in the second batch (28 days), no significant differences ($p > 0.05$) were observed in ALT levels across the groups when compared to the control. Thus, both the leaf and flower-head extracts did not significantly alter ALT activity.

**Serum ALP Activity:** The impact of MESF leaf and flower-head extracts on serum alkaline phosphatase (ALP) activity is shown in Figure 3. During the first batch (14 days), group two exhibited a non-significant increase ($p > 0.05$) in ALP activity, while groups four and seven showed no significant decrease ($p > 0.05$). In contrast, group three experienced a significant increase ($p < 0.05$), and groups five and six had significant decreases. In the second batch (28 days), ALP activity increased significantly ($p < 0.05$) in groups two and three, remained unchanged ($p > 0.05$) in groups four and five, and showed non-significant decreases ($p > 0.05$) in groups six and seven compared to their respective controls. This suggests that MESF leaf extract notably enhanced ALP activity.

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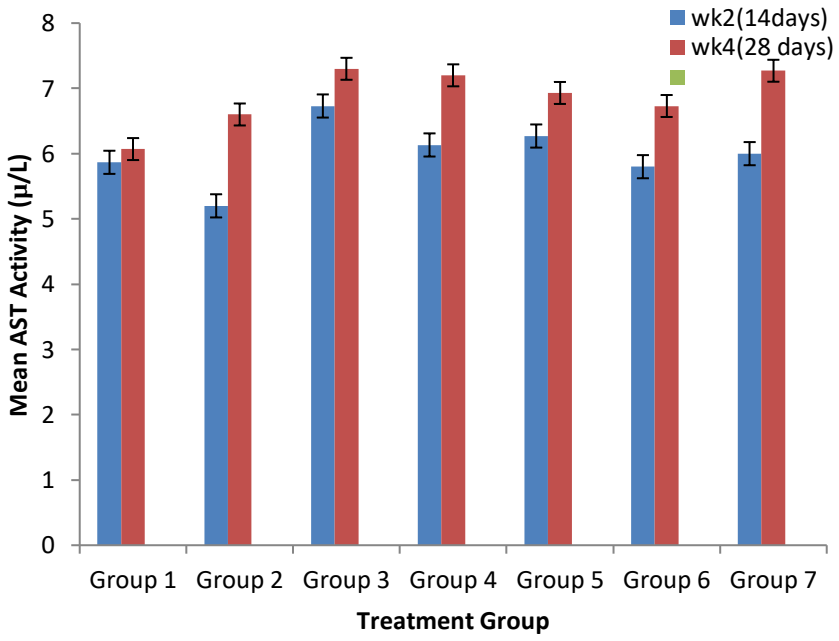


**Fig 1. Bar chart showing the effect of MESF leaf and flower-head on serum AST activity of albino rats**

Fig 1. Showed bar chart showing the effect of MESF leaf and flower-head on serum AST activity of albino rats in which Group 1 served as the control group. Groups 2, 3, and 4

were treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight (bw) of MESF leaf extract, respectively. Groups 5, 6, and 7 received 100 mg/kg, 200 mg/kg, and 400 mg/kg bw of MESF flower-head extract, respectively. Data are presented as the mean ± standard deviation (SD), with each group comprising three replicates (n = 3).

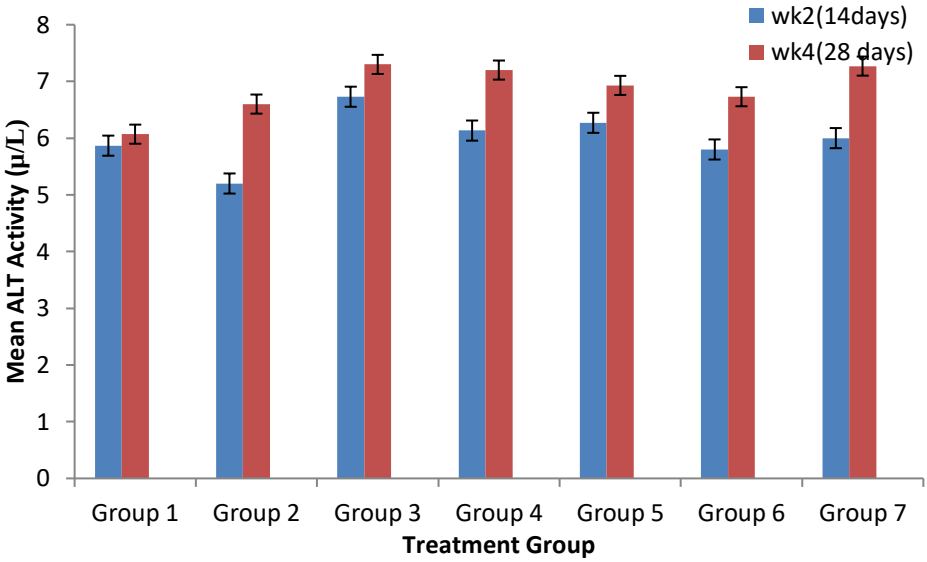


Figure 2. Bar chart showing the effect of MESF leaf and flower-head on serum ALT activity of albino rats

Figure 2. showed bar chart showing the effect of MESF leaf and flower-head on serum ALT activity of albino rats in which Group 1 was the control. Groups 2, 3, and 4 were administered MESF leaf extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight (bw), respectively. Groups 5, 6, and 7 were treated with MESF flower-head extract at the same doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg bw, respectively. Data are presented as mean ± standard deviation (SD), with each group consisting of three replicates (n = 3).

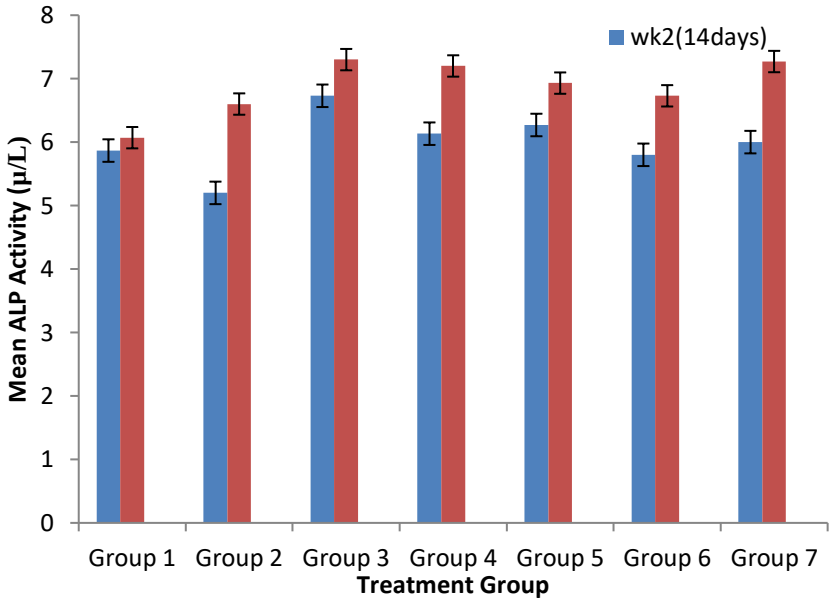


Figure 3. Bar chart showing the effect of MESF leaf and flower-head on serum ALP activity of albino rats

Figure 3 showed bar chart illustrating the impact of MESF leaf and flower-head extracts on serum alkaline phosphatase (ALP) activity in albino rats. The experimental groups are as follows: Group 1 is the control; Group 2 received 100 mg/kg body weight (bw) of MESF leaf extract; Group 3 received 200 mg/kg bw of MESF leaf extract; Group 4 received 400 mg/kg bw of MESF leaf extract; Group 5 was administered 100 mg/kg bw of MESF flower-head extract; Group 6 received 200 mg/kg bw of MESF flower-head extract; and Group 7 received 400 mg/kg bw of MESF flower-head extract. The data are presented as the mean ± standard deviation (SD), with each group comprising three replicates (n = 3).

**Effect of MESF Leaf and Flower-Head on Direct Bilirubin:** Figure 4 shows that in batch one, there was no significant decrease ($p > 0.05$) of direct bilirubin in all the groups except in group five which decreased significantly ($p < 0.05$) while in batch two, there was no significant increase ($p > 0.05$) in all the groups when compared to that in the control group.

**Effect of MESF Leaf and Flower-Head on Total Bilirubin:** Figure 5 shows that in batch one, there was no significant decrease of total bilirubin in all the groups except in group seven, while batch two shows no significant increase in all the groups when compared with their respective control groups.

**Effect of MESF Leaf and Flower-Head on Unconjugated Bilirubin:** Figure 6 shows that there was no significant increase ($p > 0.05$) of unconjugated bilirubin concentration in all the groups in batch one. In batch two, there was no significant increase ($p > 0.05$) in

groups two, three and six while groups four and seven decreased non significantly ($p > 0.05$) when compared with their control groups. There was no significant effect on the liver cells.

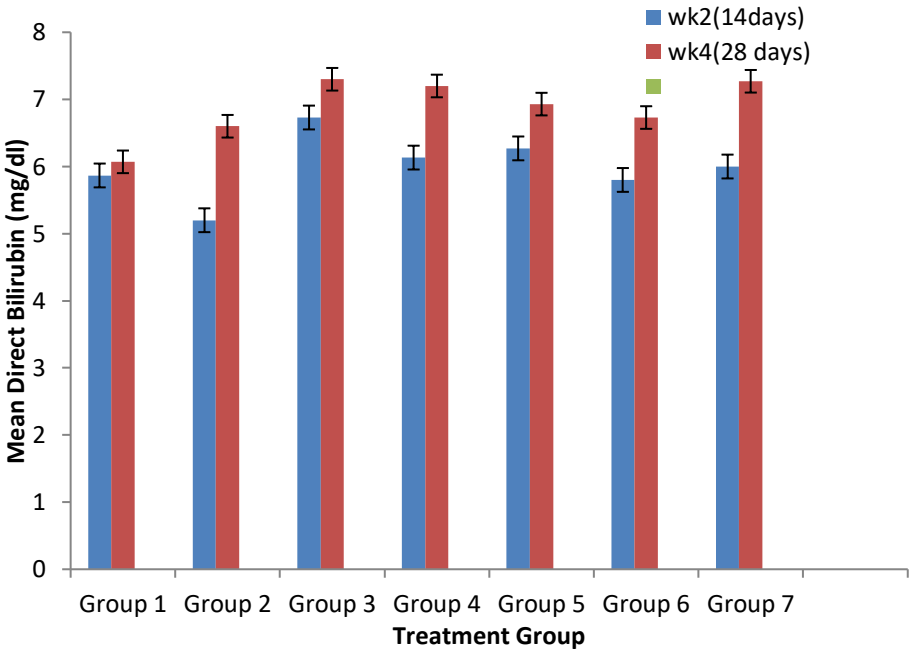


Figure 4. Bar chart showing the effects of MESF leaf and flower-head on serum direct bilirubin

Figure 4. showed bar chart depicting the effects of MESF leaf and flower-head extracts on serum direct bilirubin levels in albino rats. The groups are organized as follows: Group 1 is the control; Group 2 was treated with 100 mg/kg body weight (bw) of MESF leaf extract; Group 3 received 200 mg/kg bw of MESF leaf extract; Group 4 was administered 400 mg/kg bw of MESF leaf extract; Group 5 received 100 mg/kg bw of MESF flower-head extract; Group 6 was given 200 mg/kg bw of MESF flower-head extract; and Group 7 was treated with 400 mg/kg bw of MESF flower-head extract. Data are expressed as mean ± standard deviation (SD), with each group consisting of three replicates (n = 3).

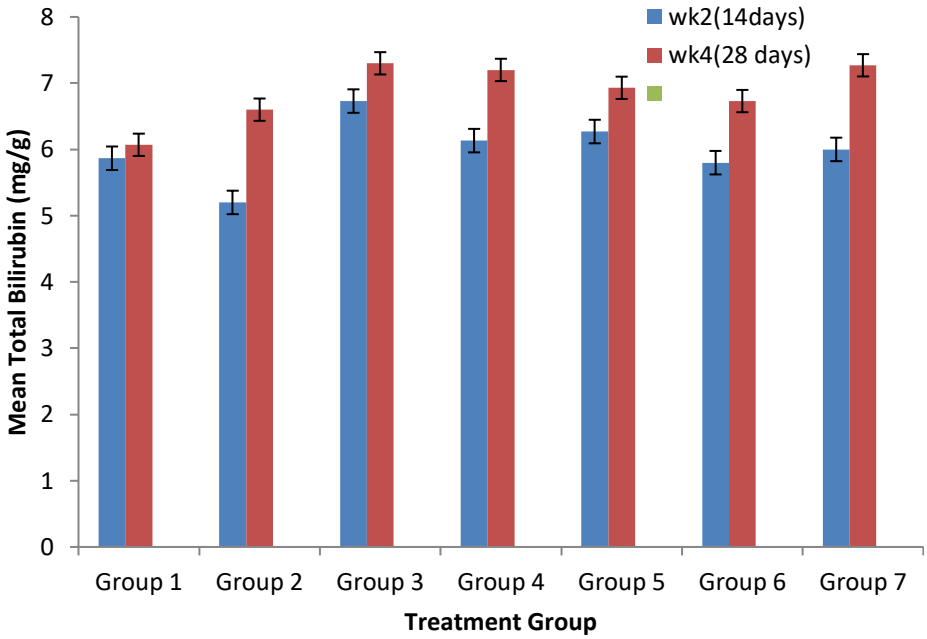


Figure 5. Bar chart showing the effects of MESF leaf and flower-head on serum Total bilirubin

Figure 5 is the bar chart illustrating the impact of MESF leaf and flower-head extracts on serum total bilirubin levels in albino rats. The groups are as follows: Group 1 serves as the control; Group 2 was treated with 100 mg/kg body weight (bw) of MESF leaf extract; Group 3 received 200 mg/kg bw of MESF leaf extract; Group 4 was administered 400 mg/kg bw of MESF leaf extract; Group 5 received 100 mg/kg bw of MESF flower-head extract; Group 6 was given 200 mg/kg bw of MESF flower-head extract; and Group 7 was treated with 400 mg/kg bw of MESF flower-head extract. The data are shown as mean ± standard deviation (SD), with each group consisting of three replicates (n = 3)..

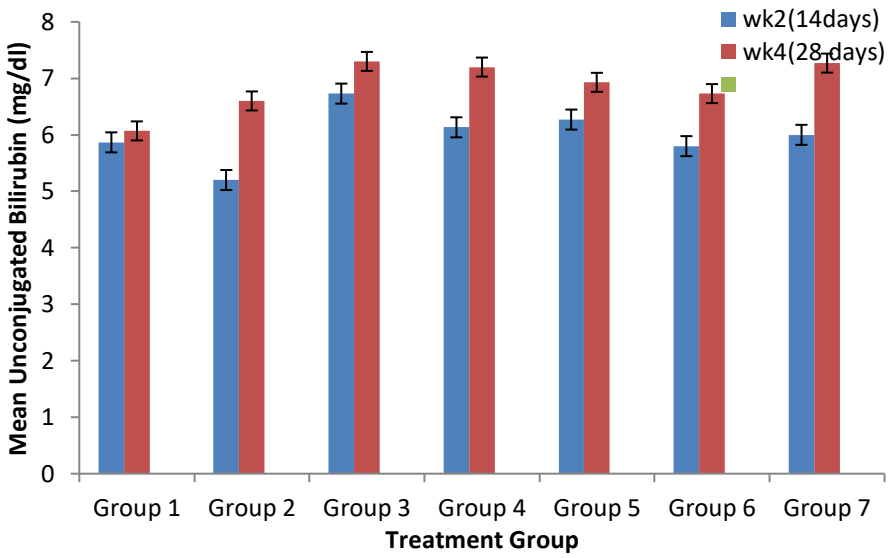


**Figure 6. Bar chart showing the effects of MESF leaf and flower-head on serum unconjugated bilirubin**

Figure 6 shows the effect of MESF leaf and flower-head extracts on serum unconjugated bilirubin levels in albino rats. The chart compares the serum levels of unconjugated bilirubin among different treatment groups, including those receiving MESF leaf extract at 100, 200, and 400 mg/kg body weight (bw), and MESF flower-head extract at the same doses, against a control group. Data are presented as mean ± standard deviation (SD) from three replicates per group. The figure helps assess how varying doses of these extracts influence unconjugated bilirubin levels, offering insights into their impact on liver function.

**Histopathology of the liver tissue**

The sections of the liver collected from the animals in the groups as represented in Figures 7, 8, 9, 10, 11, 12 and 13 did not show any significant histopathologic changes. Thus, they did not show any deviation from the normal hepatic histo-architecture.

**Histopathology on the Control Liver Cells (Group One)**

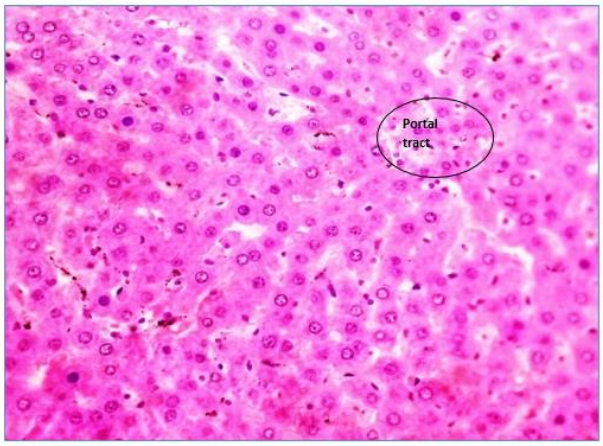


Figure.7 Photomicrograph of the liver tissue from group one rats (control) showing the portal tract. H&E × 20.

**Histopathology of Liver Tissue Fed with 100 mg/kg bw MESF Leaf (Group Two)**

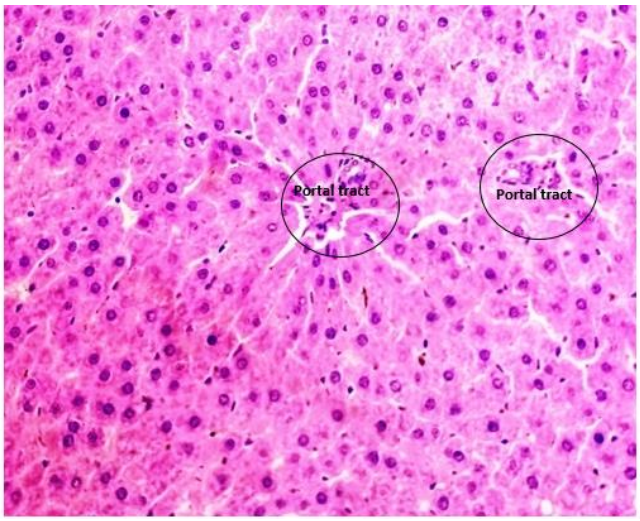


Figure 8 Photomicrograph of the liver tissue from group 2 rats fed 100 mg/kg bw of MESF leaf showing portal tracts. H&E × 20

**Histopathology of Liver Tissue Fed with 200 mg/kg bw MESF Leaf (Group Three)**

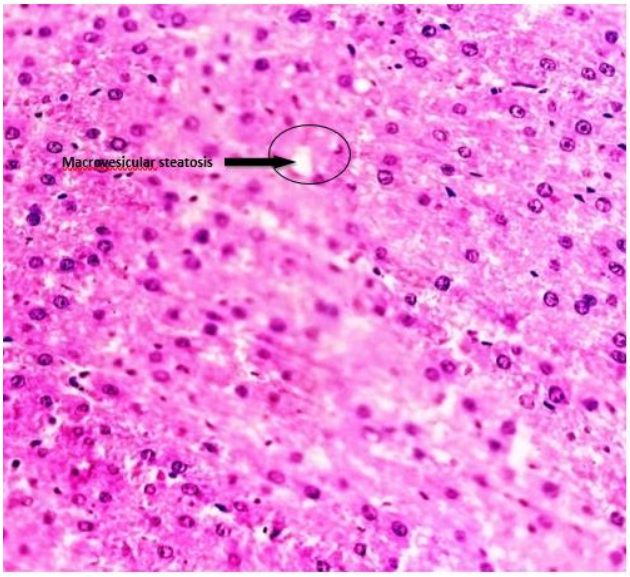


Figure 9. Photomicrograph of the liver tissue from group 3 rats fed with 200 mg/kg bw of MESF leaf exhibiting focal macrovesicular steatosis. H&E × 20

**Histopathology of Liver Tissue Fed with 400 mg/kg bw MESF Leaf (Group Four)**

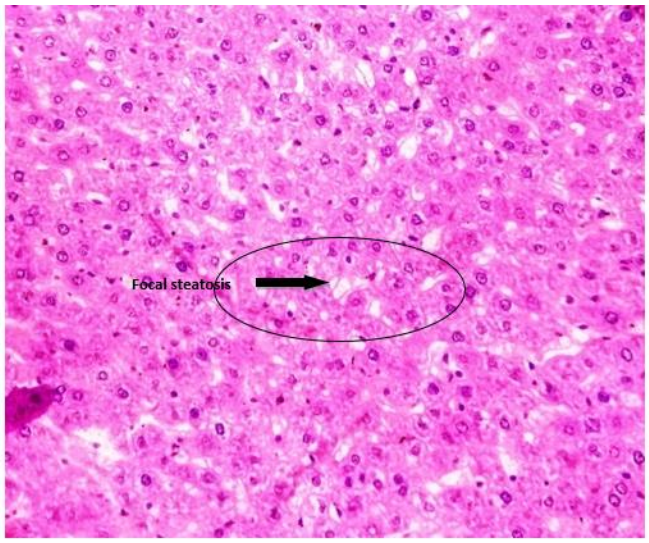


Figure 10 Photomicrograph of the liver tissue from group 4 rats fed with 400 mg/kg bw of MESF leaf showing the presence of focal steatosis. H&E × 10.

**Histopathology of Liver Tissue Fed with 100 mg/kg bw MESF Flower-Head (Group Five)**

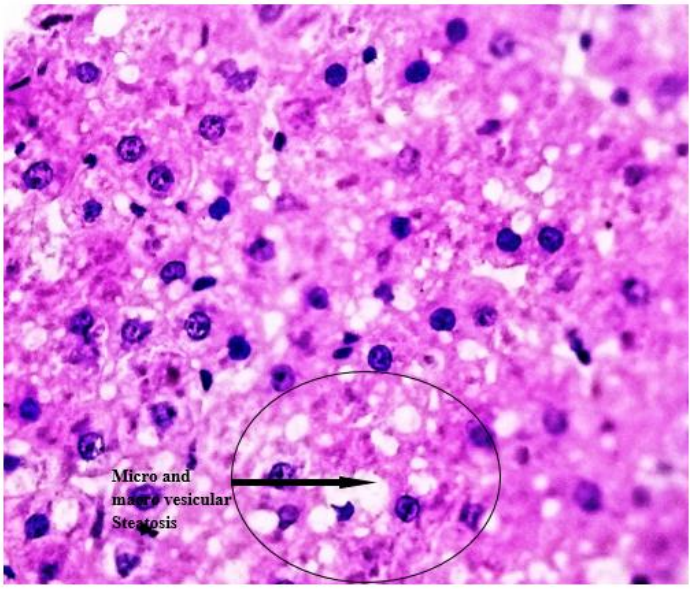


Figure 11. Photomicrograph of the liver tissue from group 5 rats fed with 100 mg/kg bw of MESF flower-head showing the presence of micro and macro vesicular steatosis. H&E × 40

**Histopathology of Liver Tissue Fed with 200 mg/kg bw MESF Flower-Head (Group Six)**

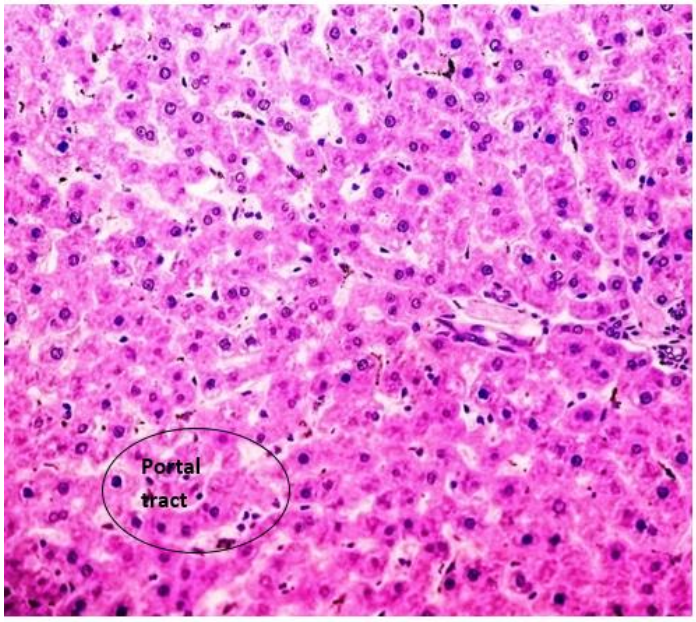


Figure 12. Photomicrograph of the liver tissue from group 6 rats fed with 200 mg/kg bw of MESF flower-head showing portal tract. H&E × 10

**Histopathology of Liver Tissue Fed with 400 mg/kg bw MESF Flower-Head (Group Seven)**

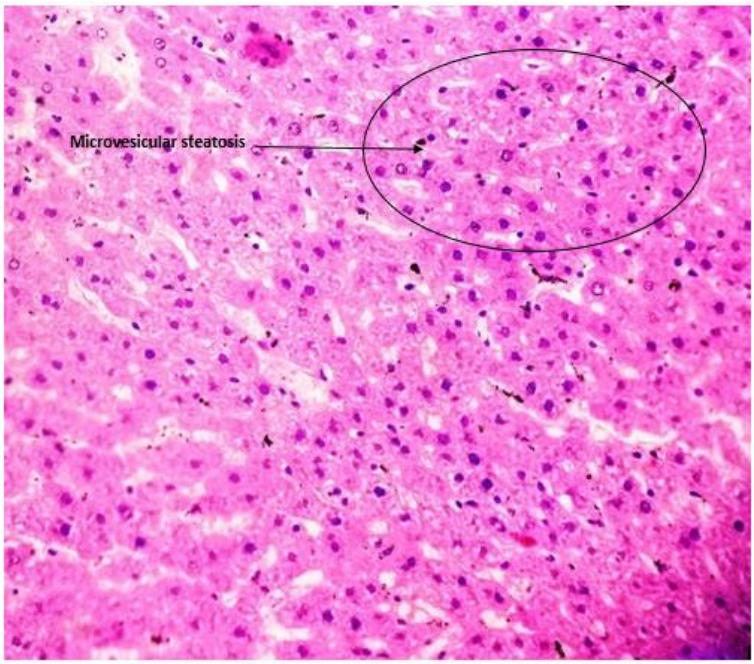


Figure 13. Photomicrograph of the liver tissue from group 7 rats fed with 400 mg/kg bw of MESF flower- head showing presence of microvesicular steatosis . H&E × 10.

**Discussion**

The evaluation of liver function and histological integrity in Wistar albino rats administered with *Spilanthes filicaulis* (MESF) flower-head and leaf extracts provided insights into the safety and potential impact of these extracts on hepatic health. The study found no significant increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in rats treated with MESF leaf or flower-head extracts (Figures 1 and 2). ALT and AST are crucial biomarkers for liver injury, as elevated levels typically indicate hepatocellular damage (Hustead and Oh, 2011). The absence of significant elevations in these enzymes suggests that the MESF extracts do not cause hepatocellular damage at the administered doses. This finding is consistent with the general understanding that significant liver injury is usually associated with elevated levels of these transaminases (Oh et al, 2017).

Furthermore, the study observed variations in alkaline phosphatase (ALP) levels, particularly in rats fed with the 100 and 200 mg/kg body weight (bw) doses of the leaf extract. ALP is an enzyme found in various tissues, including the liver, bone, kidney, and placenta (Kaplan, 1972; Riancho-Zarrabeitia et al, 2016). The increases in ALP activity noted in these groups were not uniform and did not correlate with a higher dose of the extract. Specifically, the 400 mg/kg bw dose did not result in a proportionate increase in ALP, suggesting that the observed increases in the lower doses might be due to factors

unrelated to the MESF leaf extract itself. Variability in ALP levels can result from damage to non-hepatic tissues where the enzyme is also present (Yang et al, 2024). Hence, the increase in ALP might be attributed to such tissue involvement rather than direct liver damage.

Bilirubin levels, including total, conjugated, and unconjugated bilirubin, were also assessed to provide a comprehensive view of liver function. Total bilirubin levels in the serum were not significantly altered by the treatment with MESF extracts (Figures 4 and 5). Bilirubin metabolism is a critical function of the liver, involving the conjugation of unconjugated bilirubin to make it water-soluble and its subsequent excretion (Johnston, 1999). The stability in bilirubin levels across treatment groups indicates that the MESF extracts did not disrupt bilirubin metabolism significantly. There were minor fluctuations in conjugated and unconjugated bilirubin levels, but these were not significant and did not correlate with any specific treatment group (Martelanc et al, 2014). Elevated unconjugated bilirubin typically points to issues upstream of bilirubin conjugation, such as hemolysis or liver uptake dysfunction, while elevated conjugated bilirubin suggests problems with bilirubin excretion, such as bile duct obstruction or liver pathology (Johnston, 1999). In this study, the lack of significant changes in bilirubin levels implies that MESF extracts did not induce conditions that would affect bilirubin metabolism significantly.

Histopathological examination of liver tissues provided further insights into the potential effects of MESF extracts on liver structure. Liver tissues from rats treated with both leaf and flower-head extracts did not show significant histopathological deviations from the control group (Figures 7 through 13). This includes the absence of severe hepatic injury or significant structural changes that would typically be indicative of toxic effects or chronic damage. However, some instances of macrovesicular steatosis were observed. Macrovesicular steatosis, characterized by the accumulation of fat droplets within liver cells, was noted in several treatment groups. Steatosis can result from various factors, including nutritional imbalances, metabolic disorders, or exposure to certain substances (Gök & Deveci, 2022). In the context of this study, the presence of steatosis might be linked to dietary factors rather than the MESF extracts themselves. For instance, while steatosis can be induced by liver injury or dysfunction, its presence in this study appears to be relatively mild and not accompanied by other significant histopathological abnormalities.

The specific localization of steatosis, whether periportal or perivenular, can sometimes provide additional clues about its etiology. Periportal steatosis, for instance, has been associated with conditions such as AIDS, parenteral nutrition, and kwashiorkor (Ueno et al., 1997; Sandy, & Nogueira, 2019). Since the steatosis observed in this study

was not clearly linked to any specific underlying condition or deficiency, it may be prudent to consider nutritional factors or other non-extract-related causes.

## Conclusion

The supplementation of Wistar albino rats with *Spilanthes filicaulis* (MESF) flower-head and leaf extracts did not result in significant hepatotoxicity. Biochemical assays revealed no substantial increases in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver injury. Although some variations in alkaline phosphatase (ALP) activity were observed, these changes were not consistently associated with dose or specific extract type and did not reflect liver damage as confirmed by histopathological analysis. Bilirubin levels, including total, conjugated, and unconjugated forms, remained stable across treatment groups, indicating no significant impact on bilirubin metabolism or excretion. Histopathological examination of liver tissues showed no significant deviations from normal hepatic architecture, with only minor, non-severe cases of macrovesicular steatosis noted. These findings suggest that MESF leaf and flower-head extracts are unlikely to cause significant liver damage at the administered doses, supporting their potential safety and viability for further investigation into their therapeutic applications.

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