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Research Paper

Ameliorative Effects of *Mucuna pruriens, Justicia carnea,* and Their Combined Ethanol Leaf Extracts on Biochemical Indices in Anemic Rats

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ABSTRACT

Background: *Mucuna pruriens* and *Justicia carnea* are plants with known therapeutic properties used in traditional medicine to treat a range of ailments. The present study aimed to examine the effects of *Mucuna pruriens*, *Justicia carnea*, and their combined ethanol leaf extracts on the biochemical indices of anemic rats induced by phenylhydrazine.

Methods: Thirty male albino rats were divided into six groups of five rats each. Anemia was induced using 80 mg/kg phenylhydrazine intraperitoneally, except in the normal control. Group 1 served as normal control, Group 2 as anemic control, and Group 3 received Astyfer (1.5 mg/kg). Groups 4-6 were treated with 200 mg/kg of *Mucuna pruriens*, *Justicia carnea*, and their combination. Treatments were given orally for three weeks before blood collection for biochemical analysis.

Results: Liver function test revealed a significant increase in Alkaline phosphatase level of groups 3 and 6 ($p<0.05$). Kidney function test showed a significant increase in potassium level in group 3 and urea level in groups 2 and 6 ($p<0.05$), while creatinine level significantly decreased in groups 3, 5, and 6 ($p<0.05$). Lipid profile results showed a significant decrease in total cholesterol level of groups 2, 3, and 6 ($p<0.05$). Antioxidant assay showed no significant differences ($p>0.05$), except for a significant decrease ($p<0.05$) in catalase level in group 5.

Conclusion: Findings from this study indicated that *Mucuna pruriens*, *Justicia carnea,* and their combined extract have a potent protective effect against complications arising from anemia, as the extracts restored most of the altered biochemical markers towards normal.

Keywords: Anemia, Biochemical, *Justicia carnea*, *Mucuna pruriens*, Phenylhydrazine

Introduction

Herbal drugs are substances derived from plants that are used to prevent, treat, or cure diseases. These medications can be produced from a variety of plant components, including seeds, berries, roots, leaves, bark, and flowers [1]. They are complex organic chemical combinations that may be extracted from any raw or processed plant portion [2]. Approximately 25% of medications administered globally are derived from plants [3]. Furthermore, 11 percent of the basic medicine index of the WHO is solely derived from plants, and many synthetic drugs are obtained from natural precursors [4]. The use of herbal medicine dates back more than 5,000 years, with written records indicating its use in ancient cultures. Herbal medicine was the only medicine available. Natural remedies, particularly those derived from plants, are becoming increasingly popular. Herbal medicine is employed in the treatment of a wide range of health problems, including allergies, migraines, arthritis, exhaustion, dermal diseases, lesions, scalds, digestive difficulties, and cancer. In fact, it is forecast that globally, about four-fifths of people are dependent on herbs for their basic health care, especially those in less-developed countries [5]. Most people opt for herbal medicine due to its lower cost and fewer adverse effects compared to conventional medications [6]. Whole herbs contain numerous ingredients that may be used to treat diseases and relieve symptoms [7]. The use of herbal medicine in the management of anemia has a rich history, particularly within traditional and alternative medicines such as Traditional Chinese Medicine, Ayurveda, and African traditional medicine [8].

Mucuna pruriens has received significant attention from researchers due to its rich content of L-DOPA and other bioactive compounds with substantial pharmacological and therapeutic effects. Cilia *et al.* [9] investigated the efficacy of *Mucuna pruriens* in managing Parkinson's disease. The results indicated that a single dose of *Mucuna pruriens* was non-inferior to dispersible levodopa/benserazide in terms of both efficacy and safety outcomes. High-dose of *Mucuna pruriens* showed clinical effects comparable to those of levodopa monotherapy at an equivalent dose, with

the added benefit of improved tolerability. In another study, Ayo *et al.* [10] *studied the effects of the* methanol leaf extract of *Mucuna pruriens* on anemic male rats. The results showed that oral administration of the extract significantly increased blood production in Wistar albino rats with phenylhydrazine-induced anemia. These findings suggest that *Mucuna pruriens* possesses anti-anemic properties, supporting its traditional use in folk medicine for the treatment of anemia.

Research on *Justicia carnea* has primarily focused on its traditional uses, pharmacological properties, and potential health benefits. For example, Udedi *et al.* [11] carried out research on the impact of ethanol leaf extracts of *Justicia carnea* on the reproductive function of male albino rats. Their study revealed that the extracts significantly impaired sperm dynamics and altered normal testicular histoarchitecture, with more pronounced effects observed at a dose of 200 mg/kg body weight. These findings suggest that the ethanol leaf extracts of *Justicia carnea* suppress spermatogenesis and may have potential as a contraceptive agent. The anti-anemic properties of *Justicia carnea* have been reported [12,14], validating the traditional use of the plant extracts in folk medicine for treating anemia. The study by Onyeabo *et al*. [14] demonstrated that *Justicia carnea* extract also possesses hypolipidemic properties, which may be beneficial to individuals at risk of cardiovascular disorders.

Combining plant extracts has been a common practice for improving efficacy, reducing toxicity and adverse effects, and minimizing dosage [15]. The combined extract of both plant leaves yielded enhanced hematological outcomes in anemic rats [12]. However, medicinal plants can be detrimental if administered in incorrect concentrations or using the wrong plant parts [16]. As herbal products gain global popularity, their toxicity and safety have become pressing concerns [17]. Notably, no prior study has investigated the impact of combining *Mucuna pruriens* and *Justicia carnea* leaves on key biochemical markers in phenylhydrazine-induced anemia in rats.

Materials and Methods

Sample Collection and Identification

Fresh leaves of *Justicia carnea* and *Mucuna pruriens* were picked from a garden at the Fidelity Estate in Enugu. Identification was made at the Department of Applied Biology and Biotechnology. Enugu State University of Science and Technology, and they were placed in the herbarium with voucher numbers ESUT-2024/32 and ESUT-2024/31, respectively.

Sample Preparation/Extraction

After thorough washing with distilled water, the leaves of *Mucuna pruriens* and *Justicia carnea* were air-dried at room temperature (25 ºC). The dried leaves were ground into a powder using a grinding mill. For each of the powdered leaves, 100 grams (100g) were extracted with methanol using the Soxhlet method. The resulting filtrate was concentrated at 50 ºC in a water bath. The extracts of *Mucuna pruriens* and *Justicia carnea* were mixed in an equal ratio (1:1) to create the combined dose.

Experimental Animal

Thirty male albino rats, weighing 150-200 grams, were obtained from the animal house of Nnamdi Azikiwe University, Awka, and transported to the animal house of the Department of Applied Biochemistry, Enugu State University of Science and Technology, Agbani. They were allowed to acclimatize for 7 days. The animals were put in cages with good ventilation and exposed to a 12-hour light and dark cycle at 27 °C ± 30 °C. They were given free access to water and commercial rodent chow.

Acute Toxicity Study (LD_{50})

The Up-and-Down Procedure, as outlined by Bruce [18], was used to evaluate the acute oral toxicity. A limit test using 5000 mg/kg body weight of the extracts was performed on six randomly selected rats. The animals were closely observed for 48 hours for signs of toxicity or death.

Induction of Anemia

A single intraperitoneal dose of 80 mg/kg body weight of phenylhydrazine was used to induce anemia in the rats. After 48 hours, anemia was confirmed, and blood samples were taken for biochemical analysis, using a retro-orbital sinus puncture. In order to establish baseline biochemical parameters, blood samples were obtained prior to inducing anemia.

Experimental design

Rats were divided into six groups, each consisting of five rats. Therefore:

1: was not induced or treated (normal control).

2: induced and untreated (anemic control).

3: Standard medication (Astyfer syrup–manufacturing company, Beta pharmacy) 1.5 mg/kg was administered to anemic rats.

4: Anemia-induced and given 200 mg/kg of ethanol extract of *Mucuna pruriens* (EEMP).

5: Anemia-induced and given 200 mg/kg of ethanol extract of *Justicia carnea* (EEJC).

6: Anemia-induced and given 200 mg/kg combined extract of *Mucuna pruriens* and *Justicia carnea* (EEMP + EEJC).

Treatment was given by gastric intubation once a day for three weeks.

Blood Sample Collection

After completion of treatment, all rats were sacrificed, and blood samples were collected via cardiac puncture into plain tubes. Following clotting, the samples were centrifuged using a bench centrifuge to obtain clear sera, which were used for the assays.

Liver Function Test

The liver function tests were performed using commercially available kits. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) assays were carried out using the colorimetric method described by Reitman-Frankel [19]. Alkaline phosphatase (ALP) was estimated using a kinetic colorimetric method according to the German Society of Clinical Chemistry (DGKC).

Kidney Function Tests

Urea was evaluated using the colorimetric urease-Berthelot method. Creatinine was estimated by the colorimetric method described as Jaffe's reaction. In an alkaline solution, creatinine reacts with picric acid to produce a colored complex, the intensity of which is directly proportional to the concentration of creatinine present. The electrolytes were evaluated using ion-selective electrode technology.

Lipid Profile

Cholesterol was determined using the Enzymatic Endpoint Method as outlined by Lolekha et al. [20]. Triglycerides were determined using a colorimetric method [21], while High Density Lipoproteins were estimated using a precipitation method [22].

Determination of Antioxidant Parameters

The level of Malondialdehyde (MDA) was determined by the Thiobarbituric acid reaction method [23]. Reduced glutathione (GSH) level was determined using the method of Tietze [24]. Catalase was determined using the colorimetric method of Sinha [25]. Superoxide dismutase **(**SOD**)** activity was measured by the method of Arthur and Boyne [26].

Statistical Analysis

The SPSS version 29 (SPSS Inc., Chicago, Illinois, USA) was used to analyze the collected data and displayed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the statistical significance of the results between groups, and the Tukey post hoc test was used for multiple comparisons. A $P<0.05$ was considered statistically significant.

Results

LD_{50} Test

The LD_{50} study demonstrated a wide safety margin, with no adverse behavioral effects or fatalities observed at doses of up to 5000 mg/kg body weight over a 48-hour period.

Liver Function Test

Table 1 shows that induction caused elevated ALP, ALT, and AST levels across all groups in comparison with the normal control. Groups 3 (given the standard drug) and 6 (treated with combined *Mucuna pruriens* and *Justicia carnea*) exhibited a significant increase in ALP level ($p<0.05$). No significant changes were observed in ALT and AST levels compared with the normal control ($p>0.05$).

Table 1. Effects of various treatments on the Liver function in phenylhydrazine-induced anemic rats

GROUPS	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
NC	239.88 ± 60.82	61.75 ± 15.48	119.75 ± 2.36
AC	477.48 ±24.67	72.75 ± 7.32	135.00 ± 8.49
Astyfer	553.28 ± 202.40*	60.00 ± 5.66	205.25 ± 92.18
EEMP	373.83 ± 132.86	70.25 ± 5.28	138.00 ± 22.98
EEJC	434.75 ±89.17	66.25 ± 5.74	124.00 ± 12.49
EEMP + EEJC	656.90 ± 157.81*	64.50 ± 9.11	134.50 ± 28.40

Values are mean ± SD (n=5); *-significantly increased in comparison with the normal control. NC-normal control, AC-anemic control, EEMP- ethanol extract of *Mucuna pruriens*, EEJC- ethanol extract of *Justicia carnea*, EEMP + EEJC- combined ethanol extract of *Mucuna pruriens* and *Justicia carnea.*

Effect of the Treatments on Kidney Function of the Rats

The result of the kidney function tests suggests that sodium, chloride, and bicarbonate levels remained relatively stable across all groups, with minor fluctuations. Table 2 shows a significant ($p<0.05$) increase in potassium of group 3 (treated with Astyfer) and urea level of groups 2 (anemic control) and 6 (EEMP + EEJC). In contrast, creatinine level was significantly decreased ($p<0.05$) in groups 3 (Astyfer), 5 (EEJC), and 6 (EEMP + EEJC).

Table 2. Effect of various treatments on Kidney Function in phenylhydrazine-induced anemic rats

Groups	Na (mmol/L)	K (mmol/L)	CL (mmol/L)	HCO_3 (mmol/L)	UREA (mmol/L)	CREAT (µmol/L)
NC	127.90 ± 1.19	4.93 ± 1.50	107.38 ± 1.11	15.57 ± 0.41	3.95 ± 0.37	101.43 ±7.09
AC	127.10 ± 1.40	4.89 ± 0.45	104.38 ± 1.49	18.55 ± 2.44	4.85 ± 0.26*	93.35 ± 19.71
Astyfer	128.40 ± 1.56	7.66 ± 1.21*	102.05 ± 5.29	16.32 ± 2.95	4.43 ± 0.33	73.50 ± 3.96**
EEMP	129.05 ± 2.16	5.44 ± 0.44	106.20 ± 2.87	16.07 ± 1.21	4.28 ± 0.25	94.78 ± 7.48
EEJC	128.33 ± 1.74	6.37 ± 0.40	106.28 ± 1.30	19.01 ± 2.45	3.70 ± 0.22	67.00 ± 4.74**
EEMP + EEJC	128.20 ± 0.70	4.85 ± 0.17	107.18 ± 1.37	16.19 ± 1.41	4.63 ± 0.57*	67.73 ±12.35**

Values are mean ± SD (n=5); *-significantly increased in comparison with the normal control; **- significantly decreased in comparison with the normal control; NC-normal control, AC-anemic control, EEMP- ethanol extract of *Mucuna pruriens*, EEJC- ethanol extract of *Justicia carnea*, EEMP + EEJC- combined ethanol extract of *Mucuna pruriens* and *Justicia carnea*

Effect of the Treatments on Kidney Function of the Rats

Lipid profile results showed a significant decrease ($p<0.05$) in total cholesterol level of groups 2, 3, and 6 (Table 3). Among the treatment groups, Group 4 (EEMP) maintained relatively balanced lipid levels, whereas Group 5 (EEJC) had the highest LDL levels, although the difference was not statistically significant.

Table 3. Effect of various treatments on the Lipid profile in phenylhydrazine-induced anemic rats

GROUPS	T.CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TG (mmol/L)
NC	3.10 ±0.08	1.30 ± 0.22	1.30 ± 0.43	0.48 ± 0.31	1.00 ± 0.71
AC	2.55 ± 0.06**	1.20 ± 0.14	0.95 ± 0.13	0.38 ± 0.10	0.83 ± 0.19
Astyfer	2.60 ± 0.14**	0.93 ± 0.22	1.40 ± 0.22	0.28 ± 0.10	0.48 ± 0.10
EEMP	2.95 ± 0.19	1.23 ± 0.39	1.30 ± 0.67	0.30 ± 0.08	0.68 ± 0.13
EEJC	2.90 ± 0.08	1.03 ± 0.17	1.60 ± 0.22	0.28 ± 0.05	0.60 ± 0.14
EEMP + EEJC	2.78 ± 0.05**	0.95 ± 0.13	1.53 ± 0.21	0.30 ± 0.08	0.70 ± 0.18

Values are mean ± SD (n=5); **- significantly decreased in comparison with the normal control; NC-normal control, AC-anemic control, EEMP- ethanol extract of *Mucuna pruriens*, EEJC- ethanol extract of *Justicia carnea*, EEMP + EEJC- combined ethanol extract of *Mucuna pruriens* and *Justicia carnea.*

Effect of the Treatments on the Serum Antioxidant System of the Rats

Table 4 shows that induction generally led to increased oxidative stress, as indicated by higher MDA levels and decreased antioxidant enzyme levels (GSH, SOD, Catalase) in Group 2 (Anemic control). There was no significant difference ($p>0.05$) in all the treatment groups compared to the normal control, except for a significant decrease in Catalase level in group 5 ($p<0.05$). Treatment with ethanol extract of *Mucuna pruriens* (Group 4) and the combination of *Mucuna pruriens* and *Justicia carnea* (Group 6) resulted in reduced MDA levels and improved antioxidant enzyme levels, with Group 6 showing the best improvement in overall antioxidant status. *Justicia carnea* alone (Group 5) had a moderate effect; however, the combination treatment in Group 6 was the most effective in mitigating oxidative stress.

Table 4. Effect of various treatments on the antioxidant system in phenylhydrazine-induced anemic rats

Groups	MDA(mg/dl)	GSH (Mg/dl)	SOD (U/L)	CAT(U/L)
NC	2.10 ± 0.27	16.57 ± 0.42	14.10 ± 1.16	10.19 ± 0.36
AC	4.16 ± 2.25	7.83 ± 7.68	8.42 ± 6.18	8.11 ± 2.99
Astyfer	4.22 ± 1.21	8.17 ± 5.35	9.15 ± 3.43	7.77 ± 1.18
EEMP	2.56 ± 0.49	9.40 ± 2.46	10.11 ± 1.62	7.49 ± 0.75
EEJC	2.96 ±0.07	6.40 ± 0.35	10.30 ± 0.33	6.23 ± 0.19**
EEMP + EEJC	1.69 ± 0.47	13.03 ± 1.48	12.367 ± 1.74	8.13 ± 0.21

Values are mean ± SD (n=5); **- significantly decreased in comparison with the normal control; NC-normal control, AC-anemic control, EEMP- ethanol extract of *Mucuna pruriens*, EEJC- ethanol extract of *Justicia carnea*, EEMP + EEJC- combined ethanol extract of *Mucuna pruriens* and *Justicia carnea.*

Discussion

Liver function tests are essential in evaluating the liver's health and functionality. These tests typically measure the concentrations of liver enzymes, such as ALP, ALT, and AST. These enzymes are released into the bloodstream when liver cells are injured; therefore, increased concentrations of them often signal liver damage or inflammation. The study observed changes in liver enzyme levels across five groups, including an untreated induced group and four treatment groups. After the induction of anemia, the enzyme levels in all groups significantly increased, indicating liver stress or damage. The untreated group showed persistently high levels of ALP, ALT, and AST, signifying ongoing liver dysfunction. The groups treated with *Mucuna pruriens*, *Justicia carnea*, and their combination demonstrated varying degrees of improvement in liver enzyme levels. *Mucuna pruriens* alone showed a moderate reduction in ALP and AST, indicating some hepatoprotective effects. *Justicia carnea* exhibited a more substantial reduction in ALP and AST, suggesting a stronger protective effect against liver damage. Interestingly, the combination of *Mucuna pruriens* and *Justicia carnea* resulted in the highest ALP levels, which may mean a complex interaction between the two herbs that is not entirely beneficial in reducing liver enzyme levels. Although the standard drug treatment also led to some reduction in ALT and AST levels, its effect on ALP was less pronounced compared to the herbal treatments. The findings suggest that both *Mucuna pruriens* and *Justicia carnea* have hepatoprotective properties, with *Justicia carnea* showing a slightly stronger effect. However, the combination of these herbs may require further investigation due to the unexpectedly high ALP levels observed, which could suggest potential interactions that may diminish their protective effects. These findings are consistent with earlier research indicating the hepatoprotective effects of *Mucuna pruriens* and *Justicia carnea* [27, 28].

Kidney function tests are essential for evaluating renal health and function. Key parameters include potassium (K), sodium (Na), bicarbonate (HCO_3), chloride (Cl), creatinine and urea. These measurements help assess electrolyte balance, acid-base status, and the kidney's ability to filter waste products from the blood. Abnormal levels of plasma or serum electrolytes is indicative of impaired kidney function [29]. All groups exhibited sodium levels within a narrow range, indicating that sodium balance was generally maintained across treatments. The levels were close to the baseline across the groups, with no significant deviations ($p>0.05$). The generally stable sodium and chloride levels across the groups suggest that the treatments did not significantly disrupt electrolyte balance. However, elevated potassium levels in some treatment groups warrant attention as they may indicate potential issues with potassium regulation or renal function [30]. Group 3 treated with the standard drug showed a markedly higher potassium level compared to other groups, suggesting potential disturbances in potassium homeostasis. Groups 4 and 5 also exhibited elevated potassium levels compared to the normal control group, suggesting potential effects of the treatments on potassium regulation. Elevated potassium levels in some treatment groups align with other studies, where certain treatments or herbal remedies have been shown to have effects on potassium regulation [31, 32]. Chloride levels were relatively stable among the groups, with only minor variations. This stability suggests that induction and treatments did not significantly impact chloride balance. Bicarbonate levels varied more widely. The variability in bicarbonate levels suggests that the treatments might influence acid-base balance. Group 5 had the highest HCO_3 levels, which may reflect alterations in acid-base balance due to the induction process, which could not be corrected by the treatment. Groups 2 and 3 also showed elevated bicarbonate levels, which could suggest an impact of the induction of anemia and treatment on metabolic processes. Elevated HCO_3 levels in all the groups could indicate a compensatory response to altered metabolic states [33]. The variations in creatinine and urea levels indicate differing effects of the treatments on kidney function. Urea levels were relatively consistent across groups, except for significant increases ($p<0.05$) observed in Groups 2 and 6. This consistency suggests that treatments did not cause substantial changes in urea

metabolism or excretion. Creatinine levels showed a marked variation, significantly reduced ($p<0.05$) in groups 3, 5, and 6, with Group 5 having the lowest creatinine levels, indicating potentially improved kidney filtration function and possible beneficial effects on kidney function from these treatments, which is a positive outcome.

Lipid profile tests measure various types of lipids in the blood, which are crucial indicators of cardiovascular health. Alterations in lipid levels are linked to a higher risk of cardiovascular diseases [34] and can provide insights into the effectiveness of therapeutic interventions. In this study, lipid profiles were measured across different treatment groups after an induction phase. The control group (Group 1) had balanced lipid levels, providing a baseline for comparison. After induction, most groups exhibited changes in their lipid profiles: Group 2 (anemic control: Induction + no treatment) showed reduced total cholesterol, HDL, LDL, and triglycerides compared to the control, which might reflect the effect of induction on metabolism of lipid without intervention. Group 3 (Induction + standard drug) showed a notable decrease in HDL and triglyceride levels, alongside an increase in LDL, suggesting that the standard drug had a mixed effect on lipid profiles. Group 4 (Induction + 200 mg/kg b.w *Mucuna pruriens*) exhibited an improvement in HDL and a moderate decrease in LDL and triglycerides, indicating that *Mucuna pruriens* may positively influence lipid metabolism. Group 5 (Induction + 200 mg/kg b.w *Justicia carnea*) showed a notable increase in LDL and a decrease in HDL and triglycerides, suggesting that *Justicia carnea* might have a complex effect on lipid profiles. Group 6 (Induction + 200 mg/kg b.w. Mucuna pruriens + Justicia carnea) demonstrated a decrease in HDL and an increase in LDL, similar to Group 5, with a moderate decrease in triglyceride levels. This suggests that the combination therapy may have a mixed or less predictable effect on lipid levels. The findings suggest that while individual treatments with *Mucuna pruriens* and *Justicia carnea* influence lipid profiles differently, their combination does not provide a straightforward enhancement in lipid profile improvement. *Mucuna pruriens* appears to have a favorable effect on HDL and triglycerides, which is beneficial for cardiovascular health, while *Justicia carnea* seems to increase LDL levels, potentially raising cardiovascular risk. The combination of the two herbs did not consistently improve lipid profiles and might warrant further examination to understand the interaction between the two substances.

Antioxidant assays are crucial for assessing oxidative stress and the ability of the body to mitigate reactive oxygen species (ROS). The MDA is a marker of lipid peroxidation, GSH is a key antioxidant, and SOD and CAT are enzymes that play vital roles in the detoxification of ROS [35]. The antioxidant assay in this study showed no significant difference ($p>0.05$) except for a significant decrease in CAT level in group 5 ($p<0.05$). However, some variations are worthy of note. Elevated MDA levels in Groups 2 and 3 suggest increased oxidative stress and lipid peroxidation in these groups. In contrast, Groups 4 and 5 had lower MDA levels, indicating less oxidative damage. The lowest MDA level was observed in Group 6, which may imply effective antioxidant activity from the treatment. Group 6 had the highest GSH levels, indicating a robust antioxidant defense. Group 2 showed markedly reduced GSH levels, suggesting compromised antioxidant defenses and higher oxidative stress. Groups 3 and 4 also showed relatively high GSH levels, indicating effective antioxidant support from the treatments. The SOD activity was highest in Group 6, indicating a strong response to oxidative stress. Groups 2 and 3 had lower SOD activity, which might reflect reduced antioxidant capacity or increased oxidative stress. Groups 4 and 5 had moderate to high SOD activity, suggesting some level of antioxidant protection. The CAT activity was highest in Group 6, indicating effective detoxification of hydrogen peroxide. A significantly lower CAT activity was seen in Group 5, suggesting potential impairments in oxidative stress management ($p<0.05$). Therefore, from the antioxidant assay, the lower MDA levels and higher antioxidant enzyme activities in Group 6 treated with the combined extracts suggest that the combined treatment of *Mucuna pruriens* and *Justicia carnea* was particularly effective in mitigating oxidative stress. This implies that the synergistic effects of these treatments could enhance antioxidant defenses more effectively than single treatments or controls.

Conclusion

Based on the obtained findings, the combination of *Mucuna pruriens* and *Justicia carnea* showed mixed results in some parameters, particularly in liver function and lipid profiles. Future studies should explore the mechanisms behind these interactions to optimize the therapeutic effects and mitigate any potential adverse interactions. The individual treatments with *Mucuna pruriens* and *Justicia carnea* demonstrated beneficial outcomes. Further studies are required to identify the bioactive compounds necessary for their effects and to evaluate the safety and efficacy of combined ethanol leaf extracts of *Mucuna pruriens* and *Justicia carnea* for human use.

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