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Original Article

Effects of Aqueous Extract of *Ficus Exasperata* (Sandpaper Leaf) on Haematological and Some Inflammatory Markers in Wistar Rats Induced Hypertension

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ABSTRACT

Hypertension is a global health concern associated with cardiovascular diseases. *Ficus exasperata* has gained popularity as an alternative therapy to manage hypertension due to its perceived safety and availability. This study evaluated some haematological and inflammatory markers of *Ficus exasperata* leaf extract in salt-induced hypertensive Wistar rats. Twenty Male Wistar rats were randomly divided into five groups: control, salt-induced + Nifedipine, salt-induced + *Ficus exasperata* leaf extract (200 mg/kg), salt-induced + *Ficus exasperata* leaf extract (400 mg/kg), and *Ficus exasperate* leaf extract (600 mg/kg). Hematological parameters were analyzed using a Hematological autoanalyzer (SYSMEX 1000), Albumin was analyzed using Bromocresogreen (BCG), and C-reactive protein was analyzed using the ELISA method. Data analysis was performed using SPSS version 25, with statistical significance set at $p < 0.05$. RBC (Red Blood Cell), PCV (Packed Cell Volume), and Hb (Hemoglobin) significantly increased upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt compared to control at $p<0.05$. However, the red cell parameters significantly decreased after administering 400 mg/kg and 600 mg/kg body weight of *Ficus exasperata* at $p < 0.05$. Albumin increased after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract compared to control at $p<0.05$ but decreased after administering 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt compared to control at $p < 0.05$. There was no significant difference in WBC (White Blood Cell) and CRP (C-reactive protein) among the groups $p > 0.05$. Conclusively, *Ficus exasperata* leaf extract, particularly at a dose of 200 mg/kg body weight, holds potential as a natural therapy for mitigating salt-induced hypertension and its associated complications.

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Introduction

Hypertension is a chronic medical condition affecting millions of people globally (Katherine *et al.,* 2021). It is characterized by persistent elevated blood pressure levels above the normal range of 120/80 mmHg. Hypertension has been noted to be a major risk factor for cardiovascular diseases such as stroke, heart attack, and heart failure (Katherine *et al.,* 2021) and can be caused by a variety of factors including genetic predisposition, lifestyle choices, and underlying medical conditions (Suzanne *et al.,* 2019). According to the World Health Organization (WHO) in 2015 hypertension yearly accounted for 9.4 million deaths globally. Also, it is projected that hypertension-related deaths will increase to 1.56 billion by 2025, making it the leading cause of death and disability globally (Tabrizi*et al*., 2016). In Africa, hypertension is a growing public health concern with an estimated 46% of adults affected, and it is projected to continue to increase due to factors such as urbanization and lifestyle changes (Steven *et al.,* 2013).

Herbal medicine has been utilized for centuries in the management of innumerable diseases, including hypertension. Many patients prefer using natural remedies, including herbal medicine, to manage their hypertension (Nahida and Feroz, 2011). It is believed that herbal remedies are generally safer, more affordable, and more readily available than conventional medications (Ekor, 2014). *Ficus exasperata*, commonly known as the sandpaper tree, is a medicinal plant belonging to the family *Moraceae*. It is a perennial plant that grows widely in tropical and subtropical regions of Africa (Adeyi *et al*., 2023). The plant is known for its distinct sandpapery texture on its leaves, and its ability to withstand harsh weather conditions. In recent years, scientific studies have begun to reveal the potential of *Ficus exasperata* in the management of hypertension and related cardiovascular diseases (Adekeye *et al.,* 2020; Adeyi *et al.,* 2023).

The leaves of *Ficus exasperata* have been found to contain several bioactive compounds, including flavonoids, alkaloids, tannins, and saponins, which contribute to the medicinal properties of the plant (Ahmed *et al.,* 2012). Flavonoids are a group of naturally occurring polyphenolic compounds that have been shown to possess antioxidant and anti-inflammatory properties (Tungmunnithum *et al*., 2018). Alkaloids are nitrogen-containing compounds that have been reported to exhibit a wide range of pharmacological activities, including anti-hypertensive effects (Heinrich *et al.,* 2021). Tannins, on the other hand, are polyphenolic compounds that are known to have antioxidant and anti-inflammatory properties (Tungmunnithum *et al.,* 2018). Saponins, which are also found in the leaves of *Ficus exasperata*, have been shown to have cardioprotective effects by reducing oxidative stress and inflammation (Adetuyi *et al.,* 2022). Therefore, this study aimed to assess the hematological and CRP effects of *Ficusexasperata* leaf extract on salt-induced hypertensive rats.

Materials and Methods

Plant Identification and Ethical Approval

Leaf of *Ficus exasperata* was purchased and taken to the Botany Department, University of Ilorin for seed identification and authentication with Reference Number: UILH/001/883/2023. Ethical approval was obtained from the Human and Animal Research Ethical Committee of the Faculty of Pure and Applied Sciences, Kwara State University, Malete with Reference Number: ERC/MOH/2023/06/095.

Aqueous Leaf Extraction of *Ficus exasperata*

Fresh leaves of *Ficus exasperata* were washed with clean running tap water to remove dirt, dead materials, and other contaminants and were then air-dried at room temperature. One kilogram (1 kg) of the air-dried leaves of the plant was milled into fine powder in a waring commercial blender. The powdered leaves were macerated in distilled water and extracted twice, on each occasion with 2.5 1itres of distilled water at room temperature for 48 hours. The combined aqueous extract was concentrated to dryness under reduced pressure at 60±1^{o}C in a rotary evaporator. The resulting aqueous extract was freeze-dried; finally giving 46.18 g (i.e., 4.618% yield) of a dark-green, powdery, crude aqueous extract of *Ficus exasperata*. Aliquot portions of the crude extract residue were weighed and dissolved in distilled water for use on each day of our experiment.

Inclusion and Exclusion Criteria

Healthy Wistar rats were included.

Unhealthy Wistar rats were excluded, as well as Wistar rats with bruises.

Experimental animals

Twenty (20) male Wistar rats weighing between 160 - 250 g were obtained from the Animal House at Kwara State University, Malete. The animals were acclimatized in plexiglass cages for 14 days before experimentation. The rats were housed in a well-ventilated room at 25°C with a light/dark cycle and given standard rat chow and water *ad libitum*.

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Experimental design

Twenty (20) Wistar rats were used for the analysis. The rats were divided into five (5) groups. Each group contains four (4) rats. The experiment was carried out on each of the rats in each group as follows;

Group 1: Normal Control (The Wistar rats were given feed plus water) (Table 1).

Group 2: 8% Salt + Nifedipine (The Wistar rats in this group were given feed and treated with Nifedipine by oral gavage and 8% salt to their drinking water for 4 weeks) (Table 1).

Group 3: 8% Salt+ *Ficus exasperata* Leaf Extract (The Wistar rats in this group were given feed and treated with 200 mg/kg body weight of *Ficus exasperata* leaf extract by oral gavage and 8% salt to their drinking water for 4 weeks) (Table 1).

Group4: 8%Salt+ *Ficus exasperata* Leaf Extract (The Wistar rats in this group were given feed and treated with 400 mg/kg body weight of *Ficus exasperata* leaf extract by oral gavage and 8% salt to their drinking water for 4 weeks) (Table 1).

Group 5: *Ficus exasperata* Leaf Extract (The Wistar rats in this group were given feed and treated with 600 mg/kg body weight of *Ficus exasperata* leaf extract by oral gauge for 4 weeks) (Table 1).

T**able 1: Preparation of *Ficus exasperata* Dosage for Experimental Group**

Group	Standard Dose	Animals Body Weight	Calculated Dose
A	Nil	212g	Nil
		118g	Nil
		221g	Nil
		192g	Nil
B	10mg/kg	249g	2.49mg
		233g	2.33mg
		246g	2.46mg
		250g	2.50mg
C	200mg/kg	225g	45.0mg
		207g	41.4mg
		217g	43.4mg
		220g	44.0mg
D	400mg/kg	223g	89.2mg
		211g	84.4mg
		192g	76.8mg
		213g	85.2mg
E	600mg/kg	217g	130.2mg
		234g	140.4mg
		207g	124.2mg
		197g	107.4mg

Blood Sample Collection

At the end of the 4-week treatment period, the rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected from the retro-orbital plexus of the animals into ethylene diamine tetra-acetic acid tubes for analyzing hematological parameters and then in heparinized tubes for the analysis of albumin and C-reactive protein. The plasma was prepared by centrifuging blood samples for (NCI, 2023) min at 3500 rpm using a bench top centrifuge. The plasma was then stored at -20°C for further analysis.

Laboratory Analysis

The Full Blood Count was carried out using a Hematological auto-analyzer (SYSMEX 1000), Albumin was estimated using bromocresol green method the absorbance read at 630nm and C-reactive protein was analyzed using an enzyme-linked immunosorbent assay.

Statistical Analysis

The data obtained was analyzed using IBM-Statistical Package for Social Sciences (SPSS) version 25.0. One-way analysis of variance (ANOVA) was used to compare the means between the groups. The data was

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expressed as means ± standard error of the mean (SEM). A p-value of < 0.05 was considered statistically significant.

Results

Red Blood Cell Count significantly fell in rats treated with Nifedipine + 8% salt compared to normal control. The Red Blood Cell Count was raised upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and consistently dropped as the rats were administered with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and then 600 mg/kg body weight of *Ficus exasperata* leaf extract. There was significant variation in red blood cell count in treatment groups compared to normal controls at p<0.05 (Table 2).

Eosinophils were significantly reduced in rats given Nifedipine + 8% salt compared to normal control, which was consistent upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt. However, there were sharp increases in eosinophils after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract (Table 2).

There was a significant reduction in monocytes in rats administered Nifedipine + 8% salt compared to normal control. The monocyte further reduces after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt, which then slightly increases after treatment with 600 mg/kg body weight of *Ficus exasperata* leaf extract. There was a significant reduction in monocytes in treatment groups compared to normal controls at p<0.05. Other hematological parameters were insignificantly different across all groups (Table 2)

Table 2: Haematological Parameters of Wistar Rats Treated with Different Concentrations of *Ficus Exasperata* Leaf Extract

Parameters	Group 1 Mean ± SEM	Group 2 Mean ± SEM	Group 3 Mean ± SEM	Group 4 Mean ± SEM	Group 5 Mean ± SEM	P-Value
RBC (×10^{12}/L)	6.41±0.09a	5.69±0.08b	6.67±0.07c	6.25±0.02d	5.62±0.01b	0.000**
HB (g/dl)	12.83±2.19a	11.58±0.16a	12.54±0.85a	12.50±0.43a	11.23±0.62a	0.236
PCV (%)	38.50±6.56a	34.75±0.50a	38.75±3.77a	37.5±1.29a	33.75±1.89a	0.216
MCV (fl)	59.99±0.02a	61.05±1.14a	58.47±2.98a	59.98±0.03a	60.10±0.16a	0.208
MCH (Pg)	20.01±0.00a	20.35±0.38a	18.99±2.00a	19.99±0.01a	20.00±0.10a	1.271
MCHC (g/dl)	33.34±0.01a	33.34±0.03a	32.46±1.77a	33.34±0.01a	33.28±0.12a	0.955
WBC (×10^{9}/L)	4.65±0.91a	4.5±1.92a	5.03±3.06a	5.20±1.21a	5.13±0.39a	0.123
NEU (%)	37.25±8.02a	45.50±12.15a	31.50±10.88a	47.00±28.50a	45.00±23.45a	0.722
LYMPH (%)	61±8.37a	54.00±11.43a	68.25±10.40a	52.5±27.68a	54.25±23.07a	0.714
EOS (%)	1.33±0.58a	1.00±0.00a	1.00±0.00a	2.00±0.00b	1.50± 0.71ab	0.019**
MON (%)	1.50±0.71a	1.00±0.00b	0±0.00c	0±0.00c	1.00±0.00b	0.000**
BASO (%)	0±0.00a	0±0.00a	0±0.00a	0±0.00a	0±0.00a	1.000

Mean ± SEM with the same alphabet along the row showed no statistically significance difference at p<0.05.

Albumin was slightly reduced insignificantly in rats treated with Salt Loaded (8%) + Nifedipine compared to normal controls, which slightly increased again after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt. However, there was a sudden fall in Albumin among rats treated with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt, which rises again after treatment with 600 mg/kg body weight of *Ficus exasperata* leaf extract. Albumin was highest in normal controls and lowest in rats treated with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt. There was no significant change in Albumin levels in treatment groups compared to normal controls at p > 0.05 (Table 3).

Table 3: Albumin Level of Wistar Rats Treated with Different Concentrations of *Ficus exasperata* Leaf Extract

Parameters	Group 1 Mean±SD	Group 2 Mean±SD	Group 3 Mean±SD	Group 4 Mean±SD	Group 5 Mean±SD	P-Value
Albumin (g/dL)	33.00±1.87a	30.25±1.48ab	32.50±1.66ab	26.25±4.60b	32.75±1.79a	0.009**

Mean ± SEM with the same alphabet along the row showed no statistically significance difference at p<0.05.

The CRP increases insignificantly in rats fed with Salt Loaded (8%) + Nifedipine compared to normal controls. The CRP further increases upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt. However, there was a steady decrease in CRP after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf

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extract. CRP was highest in rats treated with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and lowest in normal controls. There was no significant change in CRP level in treatment groups compared to normal controls at $p > 0.05$ (Table 4).

Table 4: C-Reactive Protein of Wistar Rats Treated with Different Concentrations of *Ficus exasperata* Leaf Extract

Parameters	Group 1 Mean±SD	Group 2 Mean±SD	Group 3 Mean±SD	Group 4 Mean±SD	Group 5 Mean±SD	P-Value
CRP (ng/mL)	1.70±0.30a	1.89±0.51a	1.98±0.36a	1.83±0.27a	1.73±0.22a	0.440

Mean ± SEM with the same alphabet along the row showed no statistically significance difference at $p<0.05$.

Discussion

This study aimed to investigate some hematological and biochemical test effects of *Ficus exasperata* leaf extract on salt-loaded induced hypertensive rats. In the present study, Red Blood Cell Count was raised upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt, but decreased after administering 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and then 600 mg/kg body weight of *Ficus exasperata* leaf extract and it is statistically significant at p-value < 0.05. The initial increase in RBC count followed by a decrease with increasing doses of *Ficus exasperata* leaf extract is a noteworthy finding. This biphasic response suggests a complex interaction between the leaf extract, salt, and erythropoiesis. This phenomenon has been observed in other studies involving herbal extracts and plant compounds. Ginkgo biloba extract has shown an increase in RBC count due to its antioxidant and vasodilatory effects (Bhattacharya, 2009). On the other hand, the decrease in RBC count at higher doses might be attributed to potential toxicity or adverse effects associated with high concentrations of certain phytochemicals in the *Ficus exasperata* leaf extract. This dose-dependent response has been reported in studies involving other medicinal plants (Ekor, 2014).

Our study showed that Packed Cell Volume and Hemoglobin slightly increased upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt but reduced after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract and it is not statistically significant at p-value < 0.05. The changes in hemoglobin levels parallel the variations observed in RBC count, indicating a close relationship between these two parameters. The initial increase in Hb levels could be a compensatory response to maintain oxygen-carrying capacity under salt-induced hypertension. The subsequent decrease with higher doses suggests a potential interference with hemoglobin production. *Ficus exasperata* leaf extract has shown a dose-dependent effect on Hb levels in rats, with low doses leading to an increase and high doses causing a decrease (Sreelatha and Padma, 2009). The increase in PCV with low doses of *Ficus exasperata* leaf extract, followed by a decrease at higher doses, mirrors the trends observed in RBC count and Hb levels. The initial increase in PCV may be due to increased RBC count, contributing to higher blood viscosity, while the subsequent decrease could be attributed to the negative effects of higher doses of the leaf extract on erythropoiesis. Changes in PCV have implications for blood flow and cardiovascular health. Elevated PCV can increase the risk of thrombosis and hypertension, while decreased PCV may impair oxygen delivery to tissues. This study also showed that MCV, MCH, and MCHC vary in treatment groups compared to normal controls. The lowest value of the red cell indices was recorded in rats treated with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and it is not statistically significant at p-value > 0.05. Variations in red cell indices might provide insights into the size, hemoglobin content, and concentration of red blood cells. These changes suggest potential alterations in erythropoiesis. To fully understand the significance of these variations, further research is needed to explore the underlying mechanisms. Fluctuations in red cell indices can be suggestive of anemia or other hematological conditions (Bain, 2015).

The present study showed that Neutrophils sharply dropped after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt but increased again after administration of 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract. Lymphocytes shoot up upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt but suddenly dropped again after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract. There is a significant reduction in eosinophils level in rats treated with 200 mg/kg body weight of Ficus and rats treated with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt at p-value < 0.05. However, there were sharp increases in eosinophils after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus*

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exasperata leaf extract and it is statistically significant at p-value < 0.05. There was a significant reduction in monocytes in rats after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt but increased after treatment with 600 mg/kg body weight of *Ficus exasperata* leaf extract at p-value < 0.05. The fluctuations in different types of white blood cells indicate the potential immunomodulatory effects of the leaf extract. Neutrophils and lymphocytes, for example, play essential roles in the immune response, and their variations suggest complex interactions with the immune system. *Echinacea purpurea* extract has shown dose-dependent effects on neutrophils and lymphocytes (Rininger*et al.*, 2000). The changes in eosinophils and monocytes also highlight the multifaceted nature of the immune response to the leaf extract and salt combination.

This study revealed that Albumin increased after treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract. Albumin was lowest in rats treated with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and it is statistically significant at p-value < 0.05. The CRP was stable upon treatment with 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt and 600 mg/kg body weight of *Ficus exasperata* leaf extract and it is not statistically significant at p-value> 0.05. However, there was a steady increase in CRP after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt. The changes in albumin and CRP levels have implications for nutritional status and inflammation. The increase in albumin levels at low and high doses of the leaf extract may indicate improved protein status. However, the decrease in albumin levels at a moderate dose suggests a potential adverse effect or a shift in protein metabolism. The increase in CRP levels after treatment with 400 mg/kg of the leaf extract suggests an inflammatory response. Elevated CRP is often associated with cardiovascular diseases and hypertension (Pearson *et al.*, 2003). This finding underscores the importance of studying the inflammatory response when assessing the effects of herbal extracts in hypertensive conditions.

Conclusion

The study suggests the optimal dose for mitigating the adverse effects of salt-induced hypertension in this rat model is 200 mg/kg body weight of *Ficus exasperate* leaf extract + 8% salt. At 200 mg/kg, several hematological parameters, including Red Blood Cell Count, Hemoglobin, Packed Cell Volume, Neutrophils, Lymphocytes, Eosinophils, and Monocytes, exhibited favorable changes. Albumin levels increased, and CRP remained stable, indicating potential therapeutic benefits.

Recommendations

1. It is recommended that further research investigates the mechanisms underlying the beneficial effects of 200 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt treatment. Understanding the pathways involved may provide insights into developing targeted therapies for salt-induced hypertension.
2. The steady increase in CRP levels after treatment with 400 mg/kg body weight of *Ficus exasperata* leaf extract + 8% salt indicates potential inflammation. Physicians should closely monitor CRP levels in patients undergoing this treatment and consider anti-inflammatory interventions when necessary.
3. Future research should investigate the reasons behind the adverse effects observed at higher doses (400 mg/kg and 600 mg/kg) of *Ficus exasperata* leaf extract. Understanding the dose-response relationship and potential toxicity is crucial for ensuring the safety and efficacy of this treatment in hypertensive patients.

Authors Declaration Statements

Plant Identification and Ethical Approval

A leaf of *Ficus exasperata* was purchased. The leaf was taken to the Botany Department, University of Ilorin for seed identification and authentication – Reference Number: UILH/001/883/2023. Ethical approval was obtained from Human and Animal Research Ethical Committee of the Faculty of Pure and Applied Sciences, Kwara State University, Malete.

Conflict of interest

The authors declared none

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