

**Haematological, Renal and Gastric Effects of Ethanol Leaf Extract of *Bauhinia variegata* Linn**

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**ABSTRACT**

*Bauhinia variegata* (Linn.) is a tropical plant used in many traditional systems of medicine in the treatment of a variety of diseases including renal and gastrointestinal disorders. The aim of the present study is to evaluate the haematological, renal and gastric effects of *Bauhinia variegata* ethanol leaf extract in rats. Wistar rats of either sex were divided into four groups and treated as follows; Group 1 received distilled water (10 mL/kg), Groups 2, 3, and 4 received 100, 200, and 400 mg/kg of *B. variegata* leaf ethanol extract, respectively, orally once daily for 28 days. After the 28<sup>th</sup> day, the rats were weighed and sacrificed. Blood samples were collected by cardiac puncture and used for haematological and biochemical analyses. Histopathological examination of the kidney and stomach was done following standard procedure. Result of the haematological analysis shows that ethanol leaf extract of *Bauhinia variegata* at 200 mg/kg caused a significant decrease in the serum levels of white blood cells (WBC), red blood cells (RBC), haemoglobin (Hgb), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), and platelets (PLT), while other parameters were same as that of the control group. No significant alteration in renal function parameters at all doses tested except for an increase in serum creatinine. Histological examination of the kidneys and stomach show no abnormal tissue architecture following treatment with *Bauhinia variegata* extract. These observations indicate that *Bauhinia variegata* leaf extract is relatively safe on short term administration, but be used with caution under chronic conditions.

**Keywords:** *Bauhinia variegata*, Plant extract, Haematological parameters, Renal function, Stomach

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**Introduction**

Each human kidney is believed to be about the size of a fist.<sup>1</sup> The kidneys filter excess water and waste from the blood while producing urine. Strong and viable kidneys are essential for the maintenance of a healthy body. They are responsible for the elimination of waste, excess water, and other contaminants from the bloodstream.<sup>2</sup> Toxins are accumulated in the bladder and are discharged during urination. The kidneys help to maintain the pH, salt, and potassium levels in the body.<sup>3</sup> They produce hormones that regulate blood pressure and the production of red blood cells. The kidneys also stimulate the production of a kind of vitamin D that aids calcium absorption in the body.<sup>4</sup> Renal disease impairs the kidneys' ability to adequately perform these functions. Malnutrition, brittle bones, nerve damage, and other health issues can result from kidney disease.<sup>5,6</sup> Diabetes and hypertension increase the risk of kidney injury.<sup>7</sup> Kidney diseases include infections, cysts, kidney stones, acute renal injury, and renal failure. Mescher<sup>8</sup> defines the stomach as a muscular, hollow organ found in the gastrointestinal systems of humans and many other animals, including a number of invertebrates. With its dilated appearance, the stomach is an important organ in the digestive system. In humans and many other animals, the stomach is located between the esophagus and the small intestine.

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The stomach secretes digestive enzymes and gastric acid to aid in food digestion. The pyloric sphincter controls the passage of partially digested food (chyme) from the stomach into the duodenum, where it is peristaltically transported through the intestines.<sup>9</sup>

*Bauhinia variegata* (Linn.) is a tree that grows in moist deciduous and dry mixed deciduous forests in the tropics.<sup>10</sup> It is only found in the Himalayan foothills and adjacent Siwalik ranges, as well as recent alluvial deposits in the Indo-Pakistan subcontinent.<sup>10</sup> It grows best in climates with hot, dry summers and moderate winters. It requires plenty of sunlight and enough drainage. The tree thrives in disturbed areas such as riverbanks and streams, natural thickets, and along roadsides.<sup>10</sup> It is a popular decorative plant in gardens, yards, and public parks. It is widely utilized for medicinal and livestock feed purposes by the Indian tribes, and it is also used in many old medical systems such as Ayurveda.<sup>11</sup> It has spread to other African countries and is used for a number of purposes.<sup>12</sup> Due to folkloric claims, various parts of this plant have traditionally been used to treat a variety of ailments such as heart diseases, liver diseases, diarrhea, ulcers, eye diseases, skin problems, piles, bacterial infections, edema, and hemorrhoids.<sup>13,14</sup> Many active phytochemicals, including stigmasterol, flavone glycosides, lupeol, kaempferol-3-glucoside,  $\beta$ -sitosterol, and others, have been identified from the stem and leaves of this plant.<sup>15,16</sup> The aim of this study is to evaluate the haematological, renal as well as the gastric effects of ethanol leaf extract of *Bauhinia variegata* (Linn.) in rats.

**Material and Methods***Plant collection and identification*

Fresh leaves of *Bauhinia variegata* (Linn.) were collected from the woods in June 2021 in Karu, Nasarawa State, Nigeria. The plant

material was identified and authenticated in the Department of Botany, Bingham University, Nasarawa State, Nigeria. A voucher number BU1189 was assigned.

#### Plant extraction

The plant leaves were shade dried for two weeks. The dried plant material was then crushed into coarse powder. The powdered sample (200 g) was extracted by percolation in 70% ethanol (1 L) at room temperature. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* at 40°C using a rotary evaporator. The extract was kept at -4°C until it was needed.

#### Animals

Wistar rats of both sexes were obtained from Bingham University Animal House. The animals were fed with rodent pellets purchased from Grand Cereals Limited and allowed unlimited access to water. Ethical approval for the study with reference number BU/2021/1132 was granted by the Faculty of Health Sciences Animal Ethics Committee, Bingham University. The rats were handled according to public health guidelines for the Care and Use of experimental animals (2011).

#### Study design

Twenty-four rats of either sex weighing between 174 – 257 g were randomly selected and divided into four groups of six rats each. Group 1 received normal saline (10 mL/kg), and served as the control group. Groups 2, 3, and 4 received 100, 200, and 400 mg/kg of *Bauhinia variegata* extract, respectively. The extract was administered orally once daily for 28 days. The weights of the rats were recorded at the start of the experiment and thereafter once weekly.

#### Sample collection and analysis of Haematological and Biochemical Parameters

Blood samples were collected by cardiac puncture. Blood samples were collected in EDTA sample bottles for hematological analysis and in plain bottles for biochemical analysis. The blood sample in the plain container was centrifuged at 300rpm for 10 minutes before being split into labeled containers.<sup>8</sup>

#### Kidney function test

Electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup>), creatinine, and blood urea levels were tested using diagnostic kits as indications of renal function. The aforementioned parameters were carried out in the Chemical Pathology Department, University of Jos Teaching Hospital. The rats' kidneys were removed and stored in 10% formal saline solution before being processed, sectioned, and stained with hematoxylin and eosin (H&E) according to standard procedures.<sup>12</sup>

#### Histological analysis

Sections of each rat's kidney and stomach were surgically removed, weighed, and stored in 10% formaldehyde and thereafter used for histological analysis following standard procedures.<sup>16</sup>

#### Statistical analysis

Data were expressed as means ± standard error of mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) and differences between the means of the control and treatment groups were subjected to multiple comparisons using Dunnett's post hoc test. P < 0.05 was considered significant.

## Results and Discussion

### Effects of *Bauhinia variegata* extract on feed consumption in rats

When compared to the control, *Bauhinia variegata* leaf extract at 100, 200, and 400 mg/kg significantly (p < 0.05) reduced feed consumption in the rats in the first week of administration. However, there was no significant difference in the feed consumption between the control and the treatment groups in the second, third, and fourth week (Table 1).

### Effect of *Bauhinia variegata* on relative organ to body weight ratio in rats

At 200 mg/kg dose of the ethanol extract of *Bauhinia variegata*, a slightly higher kidney to body weight ratio and stomach to body weight ratio was observed compared to the control group. No difference was observed in the organ to body weight ratio between the control group and the groups administered 100 and 400 mg/kg doses of the extract (Groups 2 and 4) (Table 2).

### Effect of *Bauhinia variegata* on hematological parameters in Wistar rats

*Bauhinia variegata* leaf extract at 100 mg/kg dose did not cause any significant alteration in all the haematological parameters, whereas at 200 mg/kg dose, the extract resulted in a significant (P < 0.05) decrease in white blood cells (WBC), red blood cells (RBC), haemoglobin (Hgb), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), and platelets (PLT) when compared to the control group. However, at 400 mg/kg, all the haematological parameters became normal except for a significant increase in platelets compared to that of the control group (Table 3). The decrease in RBC at the moderate dose of 200 mg/kg suggests that the plant may limit red blood cell production, reduce RBC lifespan, or interfere with iron utilization by the body. Anaemia, or a decreased red blood cell count, can cause exhaustion and weakness.<sup>17</sup> Low RBC counts result in a number of symptoms and health challenges because under anaemic condition, the heart tends to work harder in order to supply enough oxygen to the body cells.<sup>18</sup> The function of hemoglobin is to transport oxygen from the lungs to the blood and subsequently to all parts of the body. Myoglobin on the other hand is a protein that accepts, transports, stores, and releases oxygen in muscle cells.<sup>19</sup> There was no significant effect of the extract on the number of basophils, neutrophils, lymphocytes, or eosinophils at all the doses tested (Table 3). This suggests that the plant has no effect on the immune system.

### Effect of *Bauhinia variegata* on kidney function

Administration of *Bauhinia variegata* leaf extract to rats did not cause any significant alteration in the serum levels of K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, and urea compared to the control group except for slight increase in serum Na<sup>+</sup> at 200 mg/kg (Table 4). This observation suggests that the ethanol leaf extract of *Bauhinia variegata* may not compromise renal function. The study revealed that *Bauhinia variegata* ethanol leaf extract caused a significant (P < 0.05) increase in serum creatinine levels even at the lowest dose administered. Creatinine levels can rise in some kidney diseases due to a loss of creatinine excretory function, muscle damage, or taking drugs that interfere with the normal function of the kidneys.<sup>21,21</sup> The degradation of tissue creatinine is the most common endogenous source of creatinine.<sup>22</sup> The group that received 400 mg/kg of the extract had considerably higher serum creatinine levels than the control group suggesting a damaging effect of the extract to the kidneys at higher doses. According to Chanda *et al.*<sup>23</sup> elevated blood creatinine levels have been associated to possible renal disease.

**Table 1:** Effects of *Bauhinia variegata* leaf extract on feed consumption in rats

Treatment	Week 1	Week 2	Week 3	Week 4
DW (10 mL/kg)	398.91 ± 1.32	303.91 ± 40.05	204.45 ± 8.46	119.30 ± 12.21
BV (50mg/kg)	286.43 ± 4.54*	293.70 ± 58.67	363.55 ± 8.44	224.50 ± 55.51
BV (100mg/kg)	260.72 ± 14.60*	262.52 ± 63.59	237.20 ± 37.55	229.51 ± 4.57
BV (200mg/kg)	281.74 ± 4.52*	355.63 ± 18.87	370.40 ± 59.31	260.15 ± 11.65

BV: *Bauhinia variegata*, \*Significantly different from the distilled water (DW) Control at p < 0.05.

Gupta *et al.*<sup>24</sup> proposed in a study that an increase in serum creatinine levels could signal potential injury to the kidney's functioning nephrons. Renal failure is most likely a potential consequence when serum creatinine levels are higher than the usual value.<sup>25-28</sup> Creatinine content in serum is an excellent diagnostic test for chronic renal disease. Increased serum creatinine concentration, as reported by Mali *et al.*<sup>29</sup> and Matonet *al.*<sup>25</sup> has been regarded as a measure of nephrotoxicity assessment. Because serum urea was unaffected in this investigation, it is probable that the plant only caused minor kidney injury. Serum urea concentration, rather than serum creatinine is regarded to be a more reliable indicator of renal function.<sup>30</sup>

#### Effect of leaf extract of *Bauhinia variegata* on the histology of the kidney and stomach

There was no alteration in the histological integrity of the structure of the rat's kidney and stomach. Histological characteristics remained unchanged at all doses of the extract after a 28-day administration when compared to the control (Figures 1 and 2). This observation suggests that the ethanol leaf extract of *Bauhinia variegata* has minimal cellular effect on the kidneys. This supports findings from previous study which reported that *Bauhinia variegata* leaves have a long-term, mildly toxic effect on the kidneys<sup>16,23</sup>.

This nephro- and gastro-protective effects of *Bauhinia variegata* maybe attributed to the numerous phytochemicals such as proteins, carbohydrates, flavonoids, glycosides, triterpenes, saponins, phenols, and anthraquinones that have been found in the plant.<sup>31,32</sup> Saponins, tannins, phenols, and triterpenoids, which have antioxidant qualities, may have resulted in decreased tissue necrosis in the organs studied.<sup>33</sup> Furthermore, the nephro- and gastro-protective effects of *Bauhinia variegata* leaf extract maybe attributed to its wound healing properties. *Bauhinia variegata* stimulate wound healing through three separate mechanisms: contraction, connective tissue matrix deposition, and epithelialization. Open wounds heal by constricting; interaction

between cells and matrix results in tissue migration toward the wound's center. Matrix deposition is the process of depositing collagen, proteoglycans, and attachment proteins to create a new extracellular matrix.<sup>32,34</sup> The process by which epithelial cells bordering the wound edge or in residual skin appendages such as hair follicles and sebaceous glands lose contact inhibition and migrate into the wound area is known as epithelialization. The number of cells in the lower layers rises as migration advances, supplying more epithelial cells.<sup>33,35</sup>

#### Conclusion

*Bauhinia variegata* is a plant with many traditional medicinal uses around the world. The results from the present study indicate that *Bauhinia variegata* leaf extract is relatively safe when used at moderate doses for a short time. However, caution should be exercised when the plant is used for chronic conditions and at higher doses.

**Table 2:** Effect of 28 days administration of *Bauhinia variegata* on relative organ to body weight ratio in rats

Treatment	Relative organ to body weight ratio (%)	
	Stomach	Kidney
DW (10 mL/kg)	0.92 ± 0.065	0.52 ± 0.34
50 mg/kg	0.93 ± 0.27	0.49 ± 0.32
100 mg/kg	1.02 ± 0.51	0.63 ± 0.11*
200 mg/kg	0.94 ± 0.14	0.56 ± 0.07

\*Significantly different from the distilled water (DW) control at p<0.05.

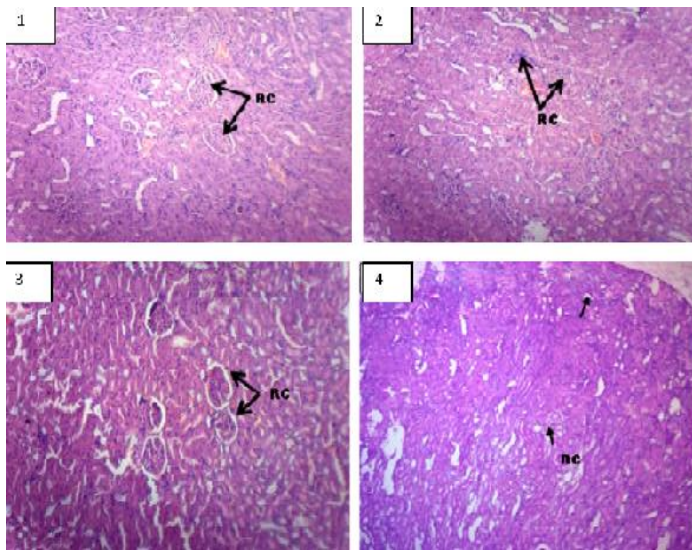
**Table 3:** Effect of ethanol leaf extract of *Bauhinia variegata* on hematological parameters in wistar rats

Haematological parameters	Treatment (mg/kg)			
	DW(10 mL/kg)	100	200	400
WBC (×10 <sup>9</sup> /L)	8.27 ± 0.72	6.75 ± 1.49	3.71 ± 0.77*	7.20 ± 1.65
RBC (×10 <sup>12</sup> /L)	8.31 ± 0.45	8.64 ± 0.64	6.13 ± 0.65*	7.74 ± 0.76
Hgb (g/dL)	15.91 ± 0.57	15.26 ± 0.66	11.56 ± 0.67*	14.58 ± 0.36
HCT (g/dL)	55.17 ± 2.07	56.64 ± 3.76	34.67 ± 3.99*	53.40 ± 1.91
MCV (fL)	66.67 ± 0.99	65.74 ± 1.435	57.17 ± 0.11*	69.60 ± 1.63
MCH (pg)	19.26 ± 0.15	17.82 ± 1.09	18.84 ± 0.77	18.82 ± 0.82
MCHC (g/dL)	29.45 ± 0.18	27.53 ± 1.23	32.55 ± 0.90*	27.11 ± 0.97
PLT (×10 <sup>9</sup> /L)	620.85 ± 52.91	567.10 ± 95.48	252.10 ± 58.34*	670.45 ± 52.78*
LYM (%)	86.82 ± 4.62	85.03 ± 4.15	82.84 ± 5.89	86.44 ± 3.17
NEUT (×10 <sup>9</sup> /L)	10.81 ± 3.67	10.80 ± 3.68	15.41 ± 5.65	11.02 ± 3.21
EOSI (×10 <sup>9</sup> /L)	1.51 ± 0.36	2.42 ± 0.71	1.83 ± 0.42	1.26 ± 0.29
BASO (×10 <sup>9</sup> /L)	1.01 ± 0.29	2.01 ± 0.59	2.54 ± 1.51	3.32 ± 2.21

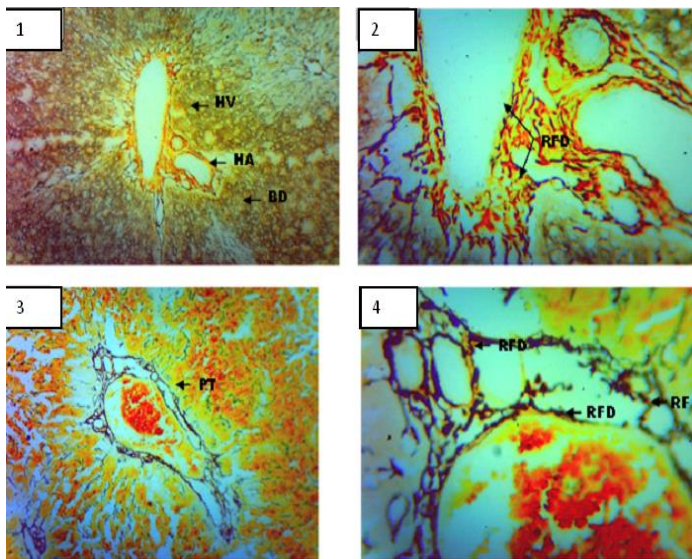
(WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils). \*significantly different from the distilled water.

**Table 4:** Effects of *Bauhinia variegata* leaf extract on kidney function

Renal indices and electrolytes	Treatment (mg/kg)			
	DW(10 mL/kg)	100	200	400
Potassium (mmol/L)	8.31 ± 0.54	9.11 ± 0.64	7.22 ± 0.82	7.55 ± 0.18
Sodium (mmol/L)	153.00 ± 2.39	163.27 ± 2.58	167.20 ± 2.13*	164.33 ± 1.82
Chloride (mmol/L)	123.12 ± 5.25	118.76 ± 6.71	126.26 ± 2.20	120.73 ± 2.54
Urea (mmol/L)	10.44 ± 0.27	10.32 ± 0.55	9.27 ± 0.20	9.67 ± 0.16
Creatinine (mmol/L)	83.13 ± 5.61	105.76 ± 5.24*	101.80 ± 7.66*	112.40 ± 9.34*



**Figure 1:** Histology of the kidney (Hematoxylin and eosin; H and E  $\times 100$ ). (1) Control group, Shows normal neurons (N), (2) 100 mg/kg, (3) 200 mg/kg, (4) 400 mg/kg of ethanol leaf extract of *Bauhinia variegata*



**Figure 2:** Histology of the stomach (Hematoxylin and eosin; H and E  $\times 100$ ). (1) Control group, Shows normal neurons (N), (2) 100 mg/kg, (3) 200 mg/kg, (4) 400 mg/kg of ethanol leaf extract of *Bauhinia variegata*.

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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