

## HISTOPATHOLOGICAL EVALUATION OF *FICUS CAPENSIS* ETHANOL EXTRACT ON LIVER, KIDNEY, INTESTINE AND ITS POTENTIALS IN TREATMENT OF ANAEMIA

### ABSTRACT

*Ficus capensis* is a native of Nigerian multi-ethnic group and it has been identified and called differently by ethnic groups for example, Igbo call it akporo, Hausa -uwaryara, etc. The aim of this research titled; Histopathological evaluation of *Ficus capensis* ethanol extract on liver, kidney, intestine and its potentials in the treatment of anaemia is to evaluate the effect of leaf and stem bark extract of *Ficus capensis* in the treatment potential of anaemia and its histopathological effect on liver, kidney, and large intestine of Wistar rat. The animals were grouped into five groups (A, B, C, D & E) and anaemia induced with cirfuroxime in all the groups except group E which is the negative control whereas other groups were treated with vitamins and leaf & stem bark of *Ficus capensis* ethanol extract; group C wasn't treated and was regarded as positive control. The blood samples were collected using cardiac puncture, and the liver, kidney and large intestine were harvested upon 14 days treatment and experimental termination following dissection upon 14 days treatment and experimental termination. All the collected samples were analysed using standard methods. There was no significant difference in body weight, same were observed in multiple comparison test on packed cell volume and haemoglobin after treatment with vitamins, leaf and stem bark of *Ficus capensis* ethanol extract. It was also observed that there was relative distortion of large intestinal lumen, kidney and hepatocytes. *Ficus capensis* leaf and bark has shown antianemic potentials however; its toxic effect on the selected organs (liver, kidney and large intestine) called for caution among users.

**Comment [pc1]:** This sentence should be improved by dividing it into two small sentences.

**Keywords:** *histopathological evaluation, ficus capensis, liver, kidney, intestine, treatment, anaemia*

## INTRODUCTION

Nature has been a source of medicinal treatments for many years and a large percentage of the world's population depends primarily on herbal medicine derived from indigenous plants for preventing, controlling, management and alleviating various disease conditions, especially in developing countries where modern Western medicine often is unavailable or is simply too expensive for the common man to afford (Otitaju *et al.*, 2014). Plant-based systems play an essential role in the primary health care of 80% of the world's developing countries (Zolfaghari and Ghannadi, 2000). Various plants have received recognition for their beneficial effects and have been discovered to contain bioactive compounds called phytochemicals and secondary metabolites that can protect humans against diseases, while others are termed underutilized vegetables, essentially due to paucity of scientific data to back up their reported traditional uses (Kumar *et al.*, 2009).

*Ficus capensis*, locally referred to as Akokoro (Igbo), Uwaryara (Hausa), Opoto (Yoruba), Rima bichehi (Fulani) and Obada (Edo), belongs to the family *Moraceae*, and has been regarded as an underutilized plant. It is used as a vegetable in foods with blood boosting effect (Adebayo-Tayo and Odeniyi, 2012). Phytochemical studies have revealed the presence of tannins, phytates, saponins, alkaloids, terpenoids and flavonoids in the leaf extract of this plant (Adebayo-Tayo and Odeniyi, 2012). The LD50 value of the aqueous leaf extract was found to be above 5000 mg/kg in albino rats when administered orally (Njoku-Oji *et al.*, 2015). Traditionally, in Nigeria, decoctions and aqueous extract of *Ficus capensis* are used in the treatment of anemia, tuberculosis, convulsions, pains, and wounds (Olowokudejo *et al.*, 2008). It is also used in treating circumcision wounds, gonorrhoea, respiratory disorders and

**Comment [pc2]:** The sentence is too long. Can it be broken into two sentences?

emollient (Olowokudejo *et al.*, 2008). Apart from its traditional uses, other benefits of *Ficus capensis* have been reported based on modern scientific investigations which include; anti-sickling (Mpiana *et al.*, 2008), antibacterial (Oyeleke *et al.*, 2013), abortifacient (Owolabi *et al.*, 2009), immune-stimulatory (Daikwo *et al.*, 2012), antidiarrhoea (Owolab, 2013), and antioxidant (Ramde-Tiendrebeogo *et al.*, 2012).

In anemia management, the drugs used are mostly multivitamins, which do not have direct stimulatory effect on erythropoiesis (Umeokoli *et al.*, 2015). These drugs are mostly used as management therapies and have slow to moderate action (Umeokoli *et al.*, 2015), while ~~the few ones that have highly stimulatory actions as well as other curative measures like curative~~ ane stimulatory measures like bone marrow transplant and blood transfusion are expensive ~~are expensive~~ (Umeokoli *et al.*, 2015). Most Africans use *Ficus capensis* for as the treatment of anemia. Hence, it is necessary to ascertain and evaluate the actions of this plant (Umeokoli *et al.*, 2015).

The ~~general~~ aim of the study is to ~~investigate~~ determine the effect of leaf and stem bark extract of *Ficus capensis* as a ~~the~~ treatment of anemia and its histological effects on liver, kidney and large intestine of Albino rats.

## **MATERIALS AND METHODS**

### **Sample Collection and Identification**

The stem bark and leaves of *Ficus capensis* were collected at Ngbo, Ohaukwu Local Government Area of Ebonyi State. The samples were identified and authenticated by Department of Crop Science, Ebonyi State University, Abakaliki.

### **Preparation of the Aqueous Extracts of *Ficus capensis***

The leaves and stem bark were thoroughly washed and air dried at room temperature for four weeks respectively. The dried leaves and stem bark were grounded into powder using a Corona manual grinding machine. Exactly 300g each of the ground leaves and stem bark powder of *Ficus capensis* were each soaked in 1 litre of distilled water for 24hrs. The aqueous extractions were sieved using muslin cloth and filtered using Whatman no. 1 (125mm) filter paper. The filtrates were separately lyophilized (freeze dried) using a lyophilizer (freeze dryer). The aqueous extract was reconstituted with distilled water in the ratio of 1:1 before administration.

### **Test Animals**

**Comment [pc3]:** Mention whether the ethical committee approval taken or not?

A total of 25 wistar albino rats weighing between 200–260g were purchased from Department of Anatomy, Ebonyi State University, Abakaliki, Ebonyi State and used for the experiment. They were maintained and housed in cages under standard environmental conditions (27°C±3°C, 12-hour light/ dark cycle) in the Department of Biochemistry animal house, Ebonyi State University, Abakaliki. They were allowed to acclimatize with the environment for one week before use. The animals were fed Vital grower's mash pellets purchased from Vital Feed Distributor at Abakaliki, Ebonyi state. At the end of a week acclimatization period, the animals were weighed, grouped and labeled.

### **Study Design**

The animals were randomized into 5 groups of 5 rats. After the induction of anemia with cefuroxime, the animals were treated for 14 days after which blood was collected by cardiac puncture under chloroform anesthesia and used for hematological analysis. The liver, kidney and large intestine were also harvested and fixed for histological analysis. They were grouped as follows: Group A – Anemia with multivitamin treatment, Group B – Anemia with Ficus

**Comment [pc4]:** Mention the dose and frequency for the treatment

capensis leaf treatment, Group C – Anemia without treatment, Group D – Anemia with Ficus capensis stem bark treatment, Group E – Negative control.

### **Induction of Anemia and Treatment**

Anemia was induced orally in the rats using 10mg/kg body weight of cefuroxime for 7 consecutive days. Blood was collected by retro orbital sinus for hematological analysis before and after the induction of anemia to monitor the animals for the symptoms of anemia before the commencement of treatment. The animals were confirmed to be anemic on the 8th day by determining their Packed Cell Volume (PCV) and Red Cell morphology, before the commencement of treatment.

**Comment [pc5]:** Mention the value of PCV determined to confirm anaemia diagnosis.

Groups B and D were the test groups and received 100mg/kg body weight of leaf extract and stem bark extract of Ficus capensis respectively for 14 days by oral gavage. Group A was given 100mg/kg body weight of multivitamin for 14 days. Group C and E served as control, hence were given normal saline.

**Comment [pc6]:** Clarify the frequency of dose

All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals (APS, 2002).

### **Sacrifice of animals**

At the end of the experimental period, the rats were grossly observed for general physical characteristics. Rats were sacrificed by anesthesia with chloroform and a midline incision was made through the anterior abdominal wall. The liver, kidney and large intestine were

isolated from the surrounding organs and fixed in 10% formal saline for 48 hours for routine histological examination.

#### **Determination of Packed Cell Volume (PCV) and Hemoglobin Concentration.**

The sample was put in an ethylene-diaminetetra-acetic acid (EDTA) bottle. The PCV of the animals were determined by microhaematocrit method as described by Cheesbrough (2002).

The hemoglobin concentration of the animals was determined using cynometh

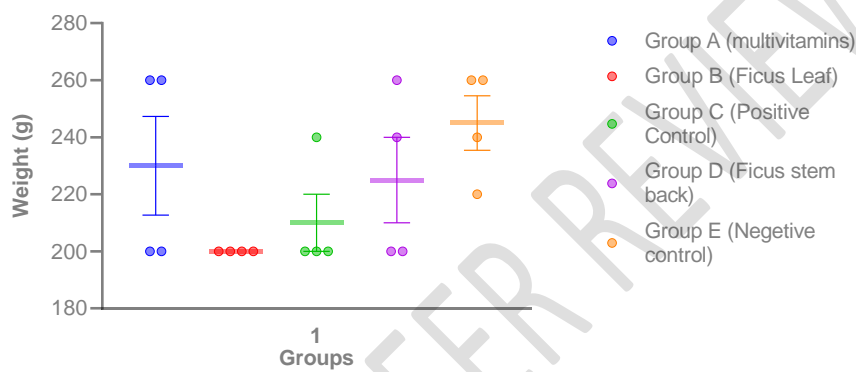
#### **Histological Procedure**

The liver, kidney and large intestine were processed via the paraffin wax embedding method of Drury and Wallington (Drury et al., 1976). Longitudinal sections of 5µm thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with Haematoxylin and Eosin (H&E) for light microscopic examination of the liver tissue architecture. Photomicrographs of the results were obtained using research photographic microscope in Alex-Ekueme Federal University Teaching Hospital, Abakaliki (AEFUTHA).

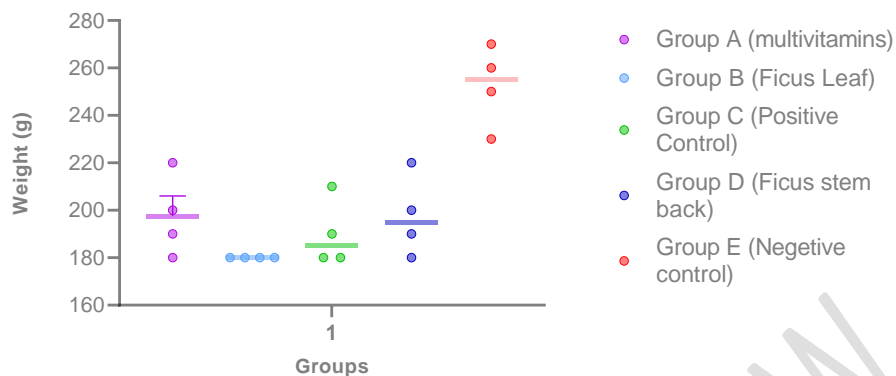
## **RESULTS**

The weights of the rats during acclimatization and during treatment of extract with multivitamins and *Ficus capensis* respectively, were recorded (Figure 1 & 2). The mean body weight of the test animals increased significantly after treatment except for group given the

Ficus leaf (Table 1). The increase in weight was observed between the negative control group and all other groups. Animals in group B given Ficus leaf extract appear to have no change in weight. The gain in weight may not be attributed to the treatment regimen, since there was no significant difference in body weight, comparing the anemic-treated (test groups) with anemic-untreated (positive control) (Table 3).



**Figure 1: Body weight of animals before treatment: Diagram showing the body weight distribution of animals used in the experiment and the mean body weight for each group.**



**Figure 2: Body weight of animals after administration: Diagram showing the body weight distribution of animals used in the experiment and the mean body weight for each group.**

**Table 1: Ordinary one-way ANOVA showing examining changes in body weight of experimental animals after administration of extract and vitamins.**

Data sets analyzed	A-E
F-value	(4,15) =14.92
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.7991

**Table 2: Tukey's multiple comparisons test showing the significant levels in body weight of experimental animals after administration of extract and vitamins**

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Sig.	Summary	Adjusted P Value	
Group A (multivitamins) vs. Group B (Ficus Leaf)	17.50	-14.52 to 49.52	No	ns	0.4695	A-B
Group A (multivitamins) vs. Group C (Positive Control)	7.500	-24.52 to 39.52	No	ns	0.9477	A-C
Group A (multivitamins) vs. Group D (Ficus stem back)	0.000	-32.02 to 32.02	No	ns	>0.9999	A-D
Group A (multivitamins) vs. Group E (Negative control)	-55.00	-87.02 to -22.98	Yes	***	0.0007	A-E
Group B (Ficus Leaf) vs. Group C (Positive Control)	-10.00	-42.02 to 22.02	No	ns	0.8667	B-C
Group B (Ficus Leaf) vs. Group D (Ficus stem back)	-17.50	-49.52 to 14.52	No	ns	0.4695	B-D
Group B (Ficus Leaf) vs. Group E (Negative control)	-72.50	-104.5 to -40.48	Yes	****	<0.0001	B-E
Group C (Positive Control) vs. Group D (Ficus stem back)	-7.500	-39.52 to 24.52	No	ns	0.9477	C-D
Group C (Positive Control) vs. Group E (Negative control)	-62.50	-94.52 to -30.48	Yes	***	0.0002	C-E

Number of families

1

Number of comparisons per family

10

Alpha

0.05

Group D (Ficus stem back) vs. Group E (Negative control)

-55.00

-87.02 to -22.98

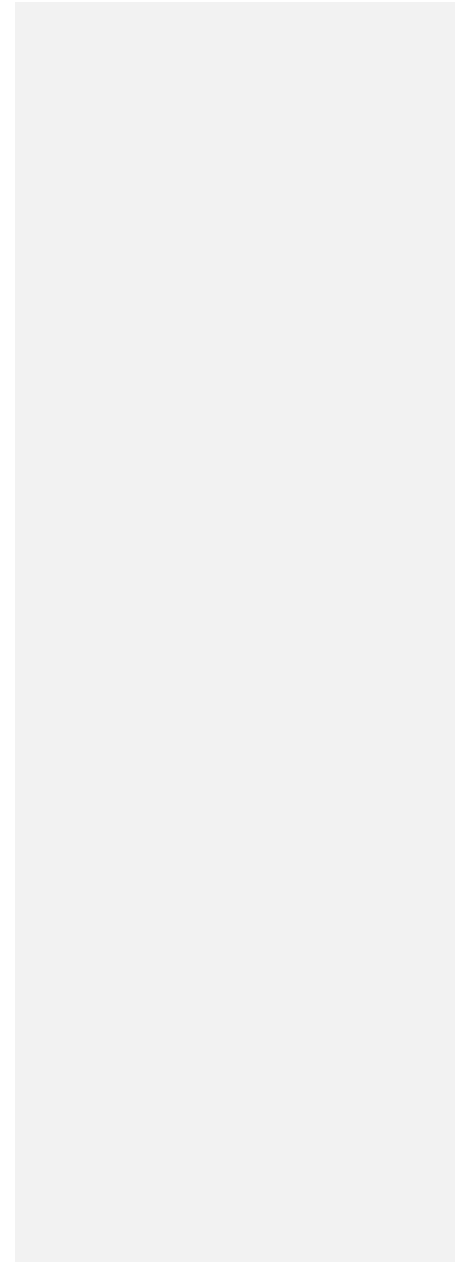
Yes

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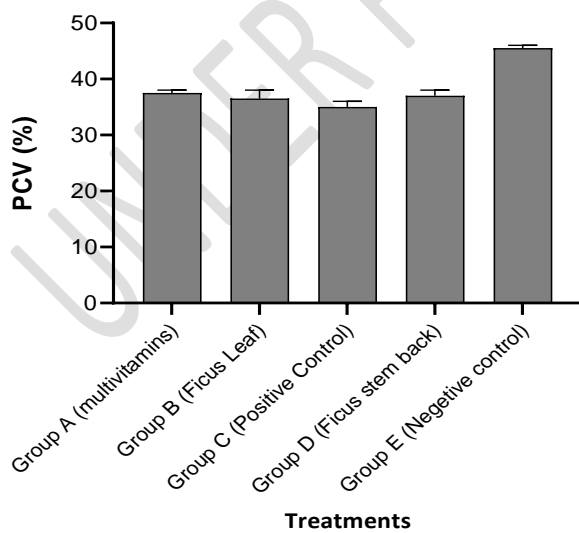
0.0007

D-E

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The result of the mean PCV of the rat after the induction of anemia ~~were~~ is shown in Table 4. There ~~is~~ was significant decrease ~~in~~ on the mean PCV of the test animals after induction of anemia with cefuroxime. After induction, there was significant difference ce between the experimental group when compared with the rats that remained uninduced (group E) ( $p < 0.05$ ) (Table 4). This suggests that cefuroxime is effective in the induction of anaemia.



**Figure 3: PCV (%) levels after induction of anaemia in experimental animals**

**Table 3: Ordinary one-way ANOVA showing examining changes in PCV (%) of experimental animals after induction of anaemia.**

<b>F</b>	DF (4,5) = 17.97
<b>P value</b>	0.0036
<b>P value summary</b>	**
<b>Significant diff. among means (P &lt; 0.05)?</b>	Yes
<b>R squared</b>	0.9350

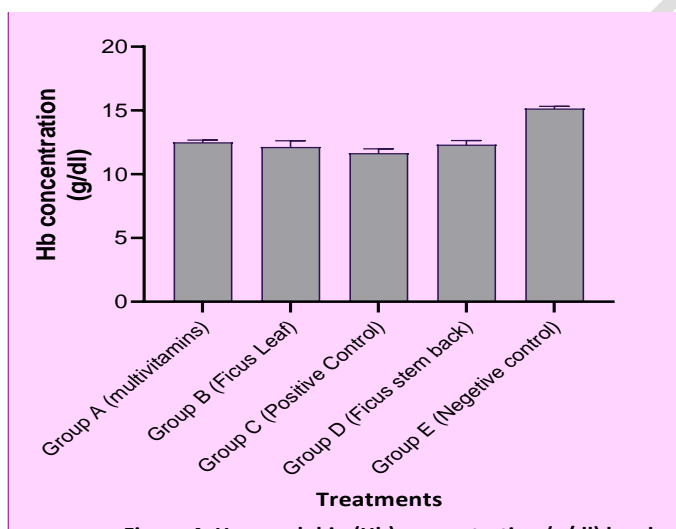
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**Table 4: Tukey's multiple comparisons test showing the significant levels of PCV (%) between treatment groups after induction of anaemia in experimental animals**

Number of families	1					
Number of comparisons per family	10					
Alpha	0.05					
<b>Tukey's multiple comparisons test</b>	<b>Mean Diff.</b>	<b>95.00% CI of diff.</b>	<b>Sign.</b>	<b>Summary</b>	<b>Adjusted P Value</b>	
Group A (multivitamins) vs. Group B (Ficus Leaf)	1.000	-4.529 to 6.529	No	ns	0.9415	A-B
Group A (multivitamins) vs. Group C (Positive Control)	2.500	-3.029 to 8.029	No	ns	0.4554	A-C
Group A (multivitamins) vs. Group D (Ficus stem back)	0.5000	-5.029 to 6.029	No	ns	0.9951	A-D
Group A (multivitamins) vs. Group E (Negative control)	-8.000	-13.53 to -2.471	Yes	*	0.0112	A-E
Group B (Ficus Leaf) vs. Group C (Positive Control)	1.500	-4.029 to 7.029	No	ns	0.8067	B-C
Group B (Ficus Leaf) vs. Group D (Ficus stem back)	-0.5000	-6.029 to 5.029	No	ns	0.9951	B-D
Group B (Ficus Leaf) vs. Group E (Negative control)	-9.000	-14.53 to -3.471	Yes	**	0.0067	B-E
Group C (Positive Control) vs. Group D (Ficus stem back)	-2.000	-7.529 to 3.529	No	ns	0.6272	C-D
Group C (Positive Control) vs. Group E (Negative control)	-10.50	-16.03 to -4.971	Yes	**	0.0033	C-E
Group D (Ficus stem back) vs. Group E (Negative control)	-8.500	-14.03 to -2.971	Yes	**	0.0086	D-E

The result of the mean Hemoglobin concentration of the rat after the induction of anemia is were shown in Table 6. There was significant decrease on the mean Hemoglobin concentration of the test animals after induction of anemia with cefuroxime. A significant difference was observed between the induced group and the negative control (group E) which were uninduced ( $p < 0.05$ ) (Table 6). Suggesting that cefuroxime is effective in the induction of anemia.

**Comment [pc7]:** Sentence needs to be restructured



**Figure 4:** Haemoglobin (Hb) concentration (g/dl) levels after induction of anaemia in experimental animals

**Comment [pc8]:** If possible mention the haemoglobin value on the bars also

**Table 5:** Ordinary one-way ANOVA showing examining changes in Hb (g/dl) of experimental animals after induction of anaemia.

**Comment [pc9]:** Title, 'showing examining' seems not proper combination

Data set analyzed	A-E
F	DF (4,5) = 19.20
P value	0.0031
P value summary	**
Significant diff. among means (P < 0.05)?	Yes 0.9389

R squared	
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**Table 6: Tukey's multiple comparisons test showing the significant levels of Hb (g/dl) between treatment groups after induction of anaemia in experimental animals**

Number of families	1					
Number of comparisons per family	10					
Alpha	0.05					
<b>Tukey's multiple comparisons test</b>	<b>Mean Diff.</b>	<b>95.00% CI of diff.</b>	<b>Sign.</b>	<b>Summary</b>	<b>Adjusted P Value</b>	
Group A (multivitamins) vs. Group B (Ficus Leaf)	0.3550	-1.432 to 2.142	No	ns	0.9211	A-B
Group A (multivitamins) vs. Group C (Positive Control)	0.8350	-0.9525 to 2.622	No	ns	0.4299	A-C
Group A (multivitamins) vs. Group D (Ficus stem back)	0.1800	-1.607 to 1.967	No	ns	0.9926	A-D
Group A (multivitamins) vs. Group E (Negative control)	-2.665	-4.452 to -0.8775	Yes	**	0.0098	A-E
Group B (Ficus Leaf) vs. Group C (Positive Control)	0.4800	-1.307 to 2.267	No	ns	0.8117	B-C
Group B (Ficus Leaf) vs. Group D (Ficus stem back)	-0.1750	-1.962 to 1.612	No	ns	0.9933	B-D
Group B (Ficus Leaf) vs. Group E (Negative control)	-3.020	-4.807 to -1.233	Yes	**	0.0056	B-E
Group C (Positive Control) vs. Group D (Ficus stem back)	-0.6550	-2.442 to 1.132	No	ns	0.6176	C-D
Group C (Positive Control) vs. Group E (Negative control)	-3.500	-5.287 to -1.713	Yes	**	0.0029	C-E
Group D (Ficus stem back) vs. Group E (Negative control)	-2.845	-4.632 to -1.058	Yes	**	0.0074	D-E

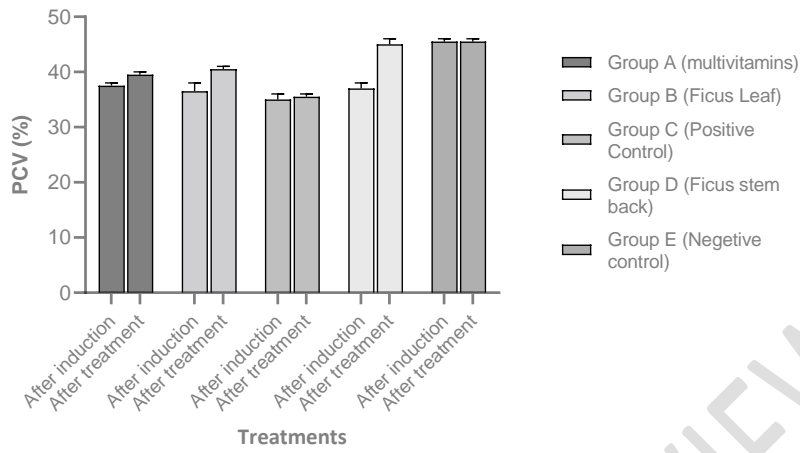
Experimental animals were treated with either with multivitamin or Ficus plant leaf or stem bark. Statistical result showed a significant change in the PCV levels of the animals when result after anaemia induction were compared to levels after treatment (Figure 5).

**Comment [pc10]:** Restructure the sentence

When the levels of significance between groups were compared, it was observed that there were changes between experimental groups except for group A and group B which did not differ from the other. Also, between group D and group E (Table 8).

The result suggest that Ficus stem bark appear to be more effective in treatment of anaemia that the Ficus leaf as the PCV level was rapidly increased. It was also observed that the Ficus leaf was as efficient as the multivitamin in the treatment of anaemia.

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**Figure 5: Comparing PCV (%) levels of experimental animals after induction of anaemia and after treatment of anaemia using Ficus plant or multivitamin.**

**Table 7: Ordinary one-way ANOVA showing examining changes in PCV (%) of experimental animals after treatment of anaemia using Ficus plant or multivitamin**

Data set analysed	A-E
F	DF (4,5) = 43.00
P value	0.0005
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9718

**Table 8: Tukey's multiple comparisons test showing the significant levels of PCV (%) between treatment groups after treatment of anaemia in experimental animals using Ficus plant or multivitamin**

Number of families	1					
Number of comparisons per family	10					
Alpha	0.05					
<b>Tukey's multiple comparisons test</b>	<b>Mean Diff.</b>	<b>95.00% CI of diff.</b>	<b>Sign.</b>	<b>Summary</b>	<b>Adjusted P Value</b>	
Group A (multivitamins) vs. Group B (Ficus Leaf)	-1.000	-4.588 to 2.588	No	ns	0.7928	A-B
Group A (multivitamins) vs. Group C (Positive Control)	4.000	0.4120 to 7.588	Yes	*	0.0329	A-C
Group A (multivitamins) vs. Group D (Ficus stem back)	-5.500	-9.088 to -1.912	Yes	**	0.0087	A-D
Group A (multivitamins) vs. Group E (Negative control)	-6.000	-9.588 to -2.412	Yes	**	0.0059	A-E
Group B (Ficus Leaf) vs. Group C (Positive Control)	5.000	1.412 to 8.588	Yes	*	0.0131	B-C
Group B (Ficus Leaf) vs. Group D (Ficus stem back)	-4.500	-8.088 to -0.9120	Yes	*	0.0205	B-D
Group B (Ficus Leaf) vs. Group E (Negative control)	-5.000	-8.588 to -1.412	Yes	*	0.0131	B-E
Group C (Positive Control) vs. Group D (Ficus stem back)	-9.500	-13.09 to -5.912	Yes	***	0.0007	C-D
Group C (Positive Control) vs. Group E (Negative control)	-10.00	-13.59 to -6.412	Yes	***	0.0005	C-E
Group D (Ficus stem back) vs. Group E (Negative control)	-0.5000	-4.088 to 3.088	No	ns	0.9759	D-E

Experimental animals were treated with either with multivitamin or Ficus plant leaf or stem bark. Statistical result showed a significant change in the haemoglobin concentration of the animals when ~~result after anaemia induction were~~ result after anaemia induction was compared to levels after treatment (Figure 6).

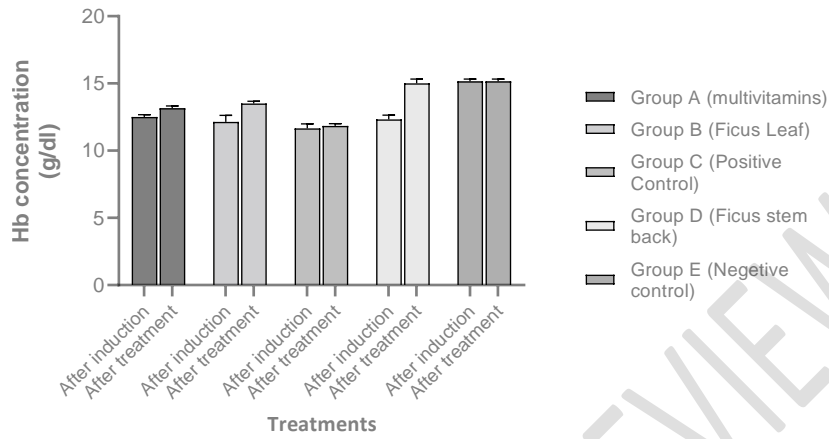
**Comment [pc11]:** Mention p value/ confidence interval for statistical significance

When the levels of significance between groups were compared, it was observed that there were changes between experimental groups except for group A and group B which did not differ from the other. Also, between group D and group E (Table 10).

The result was consistent with those obtained for the PCV further suggesting that Ficus stem bark appear to be more effective in treatment of anaemia that the Ficus leaf as the haemoglobin concentration was rapidly increased. It was also observed that the Ficus leaf was as efficient as the multivitamin in the treatment of anaemia.

**Comment [pc12]:** Mention the reference table/figure

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**Figure 6: comparing Hb (g/dl) levels of experimental animals after induction of anaemia and after treatment of anaemia using Ficus plant or multivitamin.**

**Table 9; Ordinary one-way ANOVA showing examining changes in Hb (g/dl) of experimental animals after treatment of anaemia using Ficus plant or multivitamin**

Data set analysed	A-E
F	43.49
P value	0.0004
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9721

**Table 10: Tukey's multiple comparisons test showing the significant levels of Hb (g/dl) between treatment groups after treatment of anaemia in experimental animals using Ficus plant or multivitamin**

Number of families	1					
Number of comparisons per family	10					
Alpha	0.05					
<b>Tukey's multiple comparisons test</b>	<b>Mean Diff.</b>	<b>95.00% CI of diff.</b>	<b>Sign.</b>	<b>Summary</b>	<b>Adjusted P Value</b>	
Group A (multivitamins) vs. Group B (Ficus Leaf)	-0.3350	-1.524 to 0.8536	No	ns	0.7869	A-B
Group A (multivitamins) vs. Group C (Positive Control)	1.330	0.1414 to 2.519	Yes	*	0.0324	A-C
Group A (multivitamins) vs. Group D (Ficus stem back)	-1.835	-3.024 to -0.6464	Yes	**	0.0084	A-D
Group A (multivitamins) vs. Group E (Negative control)	-2.000	-3.189 to -0.8114	Yes	**	0.0057	A-E
Group B (Ficus Leaf) vs. Group C (Positive Control)	1.665	0.4764 to 2.854	Yes	*	0.0128	B-C
Group B (Ficus Leaf) vs. Group D (Ficus stem back)	-1.500	-2.689 to -0.3114	Yes	*	0.0199	B-D
Group B (Ficus Leaf) vs. Group E (Negative control)	-1.665	-2.854 to -0.4764	Yes	*	0.0128	B-E
Group C (Positive Control) vs. Group D (Ficus stem back)	-3.165	-4.354 to -1.976	Yes	***	0.0007	C-D
Group C (Positive Control) vs. Group E (Negative control)	-3.330	-4.519 to -2.141	Yes	***	0.0005	C-E
Group D (Ficus stem back) vs. Group E (Negative control)	-0.1650	-1.354 to 1.024	No	ns	0.9762	D-E

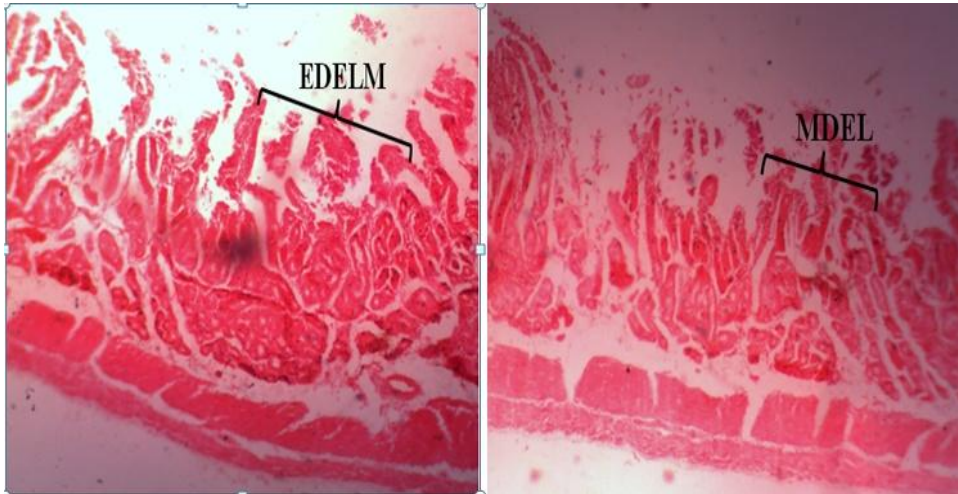


Plate 1: albino rat intestine treated with *Ficus capensis* leaf showing extensive distortion of epithelia lining of mucosa (EDELIM) and abnormal histologic architecture. Stain used: haematoxylin and eosin (Original magnification x40).

Plate 2: albino rat intestine treated with *Ficus capensis* stem bark showing mild distortion of epithelia lining of mucosa (MDEL). Stain used: haematoxylin and eosin (Original magnification x40).

The Plate A and B are the intestinal tissues of albino rats treated with *Ficus capensis* leaf and *Ficus capensis* stem bark respectively. They both shown distortion of the microvillus of the intestinal walls, erosion of the goblet cells and extensive ulceration of the secretory glands. The epithelial lining showed mild hemorrhage in *Ficus capensis* leaf treated tissues and relative red cells appearance than the tissue treated with *Ficus capensis stem bark*.

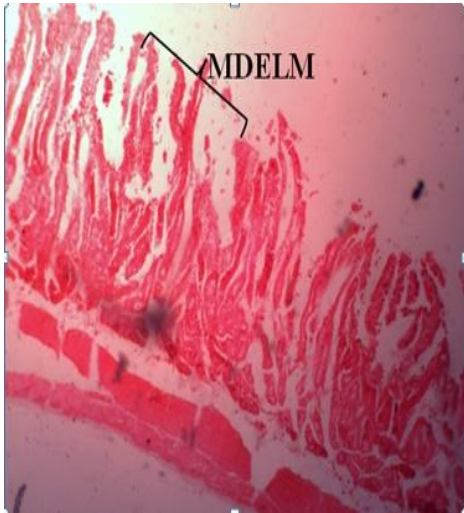


Plate 3: Albino rat intestine treated with Cefuroxime showing mild distortion of epithelia lining of mucosa (MDELM). Stain used: haematoxylin and eosin (Original magnification x40).

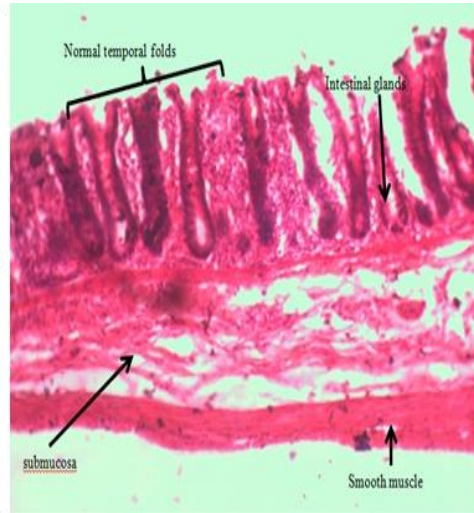


Plate 4: Negative control of Albino rat intestine, showing normal temporal folds, intestinal glands (crypts), sub mucosa (s) and smooth muscle layers (sml). Stain used: haematoxylin and eosin (original magnification x40)

Plate 3 and 4 are the albino rat tissue section treated with cefuroxime and negative control of albino rat intestine respectively. In plate 3; mild erosion of the intestinal epithelial linings which includes the villi projections and secretory glands were observed. This is contrary to plate 4; showing normal intestinal crypts, sub mucosal and smooth muscle cells. It is therefore observed that cefuroxime treatment could cause intestinal ulceration.

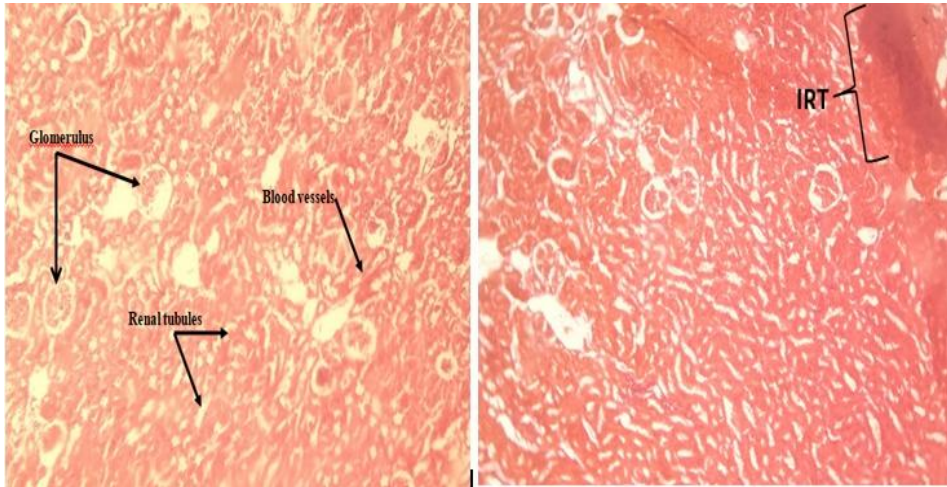


Plate 5: **Negative control of albino rat kidney, showing normal glomerulus and renal tubules. Stain used: haematoxylin and eosin (Original magnification x40).**

Plate 6: **Albino rat kidney treated with Cefuroxime showing inflammation of renal tubules (IRT). Stain used: haematoxylin and eosin (Original magnification x40).**

Plate 5 and 6 are the negative control of Albino rat kidney and albino rat kidney treated with cefuroxime respectively. plate 5; show normal glomerulus, renal tubules and blood vessels while plate 6; shown inflammation of the renal tubules (IRT). Clear renal space was observed. The inflammatory activities of the Cefuroxime treated albino rat kidney have proven that it has the capacity to induce cellular injury on the kidney tissue.

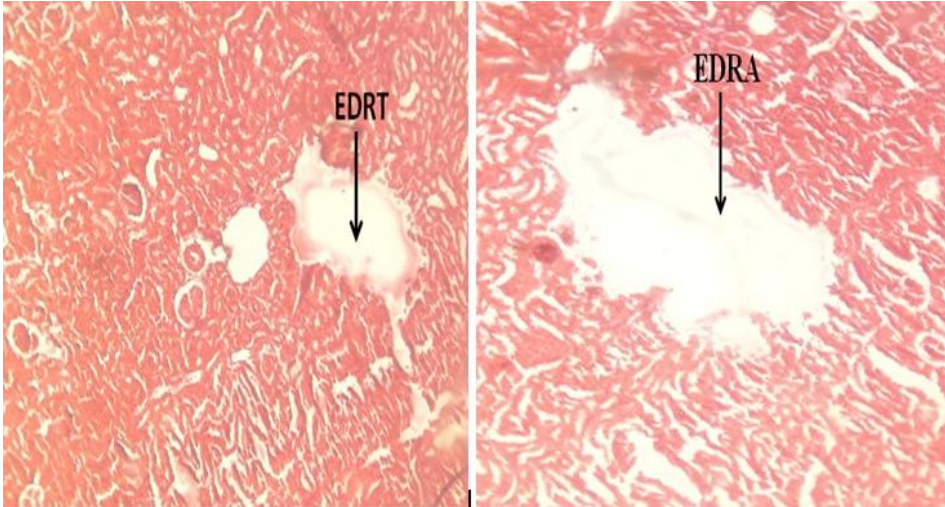


Plate 7: Albino rat kidney treated with *Ficus capensis leaf* showing extensive distortion of renal tubules (EDRT). Stain used: Haematoxylin and eosin (Original magnification x40).

Plate 8: Albino rat kidney treated with *Ficus capensis stem bark* showing an extensive distortion of renal architecture (EDRA). Stain used: Haematoxylin and eosin (Original magnification x40).

Plate 7 and 8 are the albino rat kidney section treated with *Ficus capensis* leaf and albino rat kidney section treated with *Ficus capensis* stem bark, respectively. In plate 7; Moderate to extensive distortion of renal architecture having the renal tubules damaged and necrotic cells sequel to fluid accumulation were observed. While in plate 8; severe damage of renal architecture was observed and that led to tubular damage, and necrotic area were seen following fluids accumulation. *Ficus capensis* stem bark is more lethal compared to its leaf on Kidney section.

**Comment [pc13]:** Lethal word should be replaced with more appropriate word

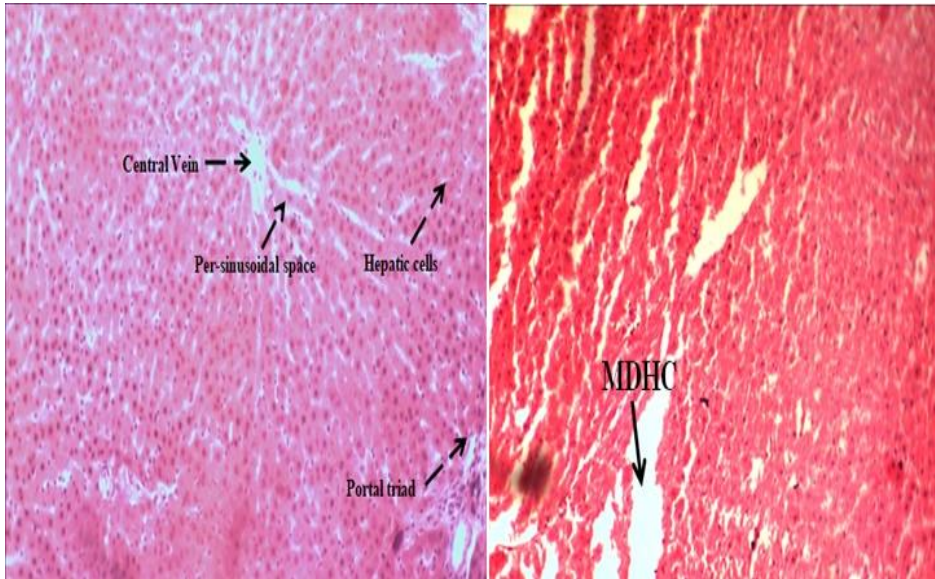


Plate 9: **Negative control of Albino rat liver showing normal central vein, hepatic cells, portal triad and peri-sinusoidal spaces (Original magnification x40).**

Plate 10: **Albino rat liver treated with Cefuroxime showing mild distortion of hepatic cells (MDHC). Stain used: Haematoxylin and eosin (Original magnification x40).**

Plate 9 and 10 are Negative control of albino rat liver and albino rat liver treated with cefuroxime respectively. plate 9; show hepatic cells, central vein and per-sinusoidal space demonstrated in normal form. In plate 10; relative inflammatory cells were observed showing minimal injury of Cefuroxime to the liver tissue and also mild distortion of Hepatic cells (MDHC) were observed.

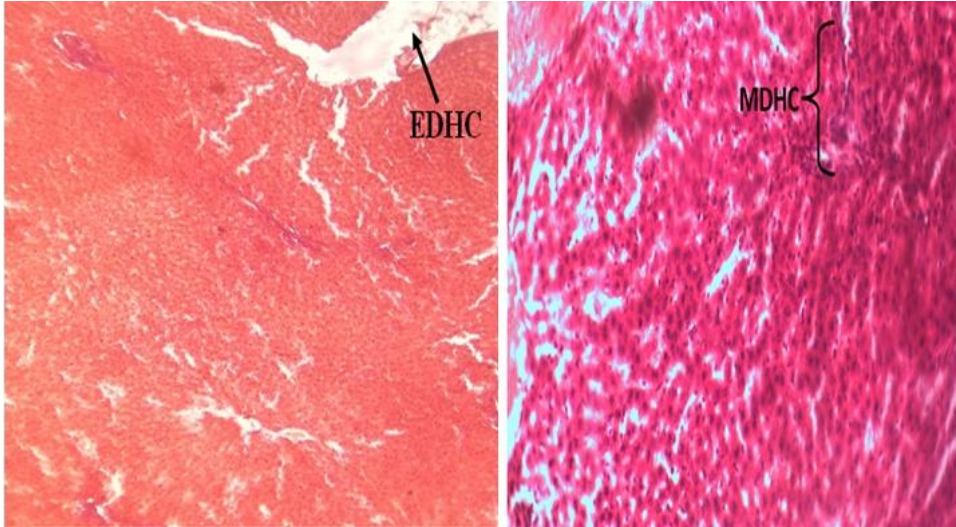


Plate 11: Albino rat liver treated with *Ficus capensis* leaf showing extensive distortion of hepatic cells (EDHC). And abnormal histological architecture. Stain used: Haematoxylin and eosin (Original magnification x40).

Plate 12: Albino rat liver treated with *Ficus capensis* stem bark showing mild distortion of hepatic cells (MDHC). Stain used: Haematoxylin and eosin (Original magnification x40).

Plate 11 and Plate 12 is the albino rat liver section treated with *Ficus capensis* leaf and the albino rat liver treated with *Ficus capensis* stem bark respectively; In Plate 11; the central vein, radiating towards the sinusoidal hepatocytes were observed with less or no damage. An extensive distortion of the hepatic (EDHC) cells was also observed. While in Plate 12; the histological section shown extensive neutrophil infiltration cum inflammatory cells. Hemorrhagic spots and mild distortion hepatic cell (MDHC) were also observed. The stem bark of *Ficus capensis* has maximum hepatic injury as observed in the above slide in plate12.

## Discussion

Anaemia is a widespread public health problem affecting about one third population, especially the poor class of the socioeconomic group in third world countries (Karine and Jennifer, 2007). The plant *Ficus capensis* is known to be used in the treatment of anemia, this brings about the question of efficacy, safety and possible toxicity. The study was carried out to ascertain the efficacy of *Ficus capensis* in treatment of anemia and the possible side effects on liver and kidney.

In the present study, administration of cefuroxime produced significant decreases in PCV and anemia in the test group compared with the control ( $p < 0.05$ ) (Table 4.3). The results from this study showed that the aqueous leaf extract and stem bark of *Ficus capensis* respectively, significantly increased the PCV and Hemoglobin concentration in the experimental animals. This is an indication that the rats recovered from anemia. Hence, it may be said that the plant extract increased the PCV and hemoglobin concentration of anaemic rats. The mechanisms behind the increase observed in packed cell volume and hemoglobin concentration in this study are not very clear. However, the presence of iron in *F. capensis* leaf extract has been reported in a previous study (Achikanu *et al.*, 2013). Iron is required to produce hemoglobin and myoglobin and has the ability to improve bone marrow functions thus increasing erythropoiesis (Orhue *et al.*, 2008). The elevated levels of the hematological parameters may also be due to the vitamin C content of the leaf extract (Achikanu *et al.*, 2013), which is required for the absorption of iron component of *F. capensis*. In addition, vitamin C prevents formation of insoluble and unabsorbable iron compounds, which in effect oppose / override the anti-iron activity of phytate and tannin components of the plant (Orhue *et al.*, 2008).

There was significant increase on the PCV and hemoglobin concentration in albino rats treated with stem bark extract of *Ficus capensis* increased when compared with those treated with leaf extract of *Ficus capensis* ( $p < 0.05$ ) (Table 8 and 10). So, it may be said that stem bark extract of *Ficus capensis* is better in the treatment of anemia than leaf extract. This may be attributed to the presence of saponins which is present in the stem bark extract and absent in the leaf of *Ficus capensis*. Saponins and their characteristic constituents ziyuglycoside I and ziyuglycoside II improve hematopoiesis by inhibiting apoptosis of TF-1 cells caused by cytokine deprivation and promote survival of bone marrow nuclear cells through focal adhesion kinase (FAK) and extracellular signal-regulated kinase 1&2 (Erk 1&2) activation (Xin *et al.*, 2017).

The histological result of the intestine showed that there was distortion of the microvillus of the intestinal walls, erosion of the goblet cells and extensive ulceration of the intestinal secretory glands of the test animals treated with *Ficus capensis* stem bark extract and leaf extract alike (Plate 1 and 2). The histological slide of the kidney showed moderate to extensive distortion of renal architecture having the renal tubules damaged and necrotic cells in rats treated with *Ficus capensis* leaf (Plate 7). Also, severe damage of renal architecture was observed and that led to tubular damage, and necrotic area were seen following fluids accumulation in the rats treated with *Ficus capensis* (Plate 8). *Ficus capensis* stem bark showed more distortion on the renal architecture, hence, could be lethal compared to the leaf on Kidney sections (Plate 5). This observed distortion in the kidneys of experimental animals may have been induced by cefuroxime for which administration of the extract could not aid recovery or this might be a slower process. The albino rat liver section treated with *Ficus capensis* leaf and the albino rat liver treated with *Ficus capensis* stem bark respectively also showed an extensive distortion of the hepatic cells. However, the stem bark of *Ficus capensis* has maximum hepatic injury as

**Comment [pc14]:** Use more appropriate terminology. Do we have estimated mortality of the extract?

observed in the above slide in plate 12. This may also have been induced by cefuroxime at the concentration used to induce anaemia. Whereas the histological appearance of the control group is consistent with normal histology.

The histopathological features observed in the liver slides of the rats treated with *Ficus capensis* stem back and leaf extract were severe degenerative changes. These liver injuries may have been caused by interference with the metabolic pathways essential for hepatocytes and parenchymal cell integrity. They lead to diversion, competitive inhibition or structural distortion of molecules essential for metabolism or to selective blockade of key metabolic pathways required to maintain the intact hepatocyte.

Comment [pc15]: Sentence is not clear

The *Ficus capensis* stem back extract contained an additional phytochemical; saponins which is termed cytotoxic indirect hepatotoxins. It induces hepatic injury by mechanisms that presumably relate to their selective interference with cell metabolism. This may be attributed to the increased tissue damage observed in the intestine, kidney and liver of rats treated with stem back extract (Plate 2, 8 & 12).

Comment [pc16]: Sentence is not clear

## Conclusion

The outcome of this study shows the haemato-stimulatory and anti-anaemic potentials of *Ficus capensis* stem back and leaf respectively and therefore can serve as haematinics. **Ficus capensis** stem back extract showed a higher anti-anaemic potential. The study also demonstrated the toxic effects of *Ficus capensis* stem back and leaf on the intestine, kidney and liver.

Hence, *Ficus capensis* leaf and stem back has anti-anaemic potentials but is very toxic to vital organs of the body.

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