ZEAL SCIENTIFIC PUBLICATION

World Journal of Advanced Pharmaceutical and Medical Research

Journal homepage: https://zealjournals.com/wjapmr/

ISSN: 2799-0656 (Online)

(Research Article)

Hematological and kidney-functional analyses of acetic acid-induced inflammatory rats administered flavonoid-rich fraction of *Ficus sur*

Sunday Adeola Emaleku *

Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

World Journal of Advanced Pharmaceutical and Medical Research, 2021, 01(01), 024–034

Publication history: Received on 09 May 2021; revised on 15 June 2021; accepted on 17 June 2021

Article DOI: https://doi.org/10.53346/wjapmr.2021.1.1.0016

Abstract

Background and aims: Unregulated inflammation cause dysfunctional immune system and could consequently lead to blood disorders due to impaired hematopoietic process, and also destruction of important organs as a result of autoimmunity. In this study, flavonoid-rich fraction of *Ficus sur* (FRFFS) is investigated for its effects on hematological parameters and renal metabolites in inflammation-induced rats.

Materials and methods: Inflammation was induced in Wistar rats of average weight of 112.60 ± 0.75 g by successive oral administration of 6% at (20 ml/kg), 3% at (15 ml/kg) and 6% at (10 ml/kg) acetic acid for nine (9) days. Experimental animals were orally administered various doses of FRFFS; 15 mg/kg, 30 mg/kg, 60 mg/kg and 120 mg/kg for two weeks. Blood and kidney samples were collected for hematological parameters and kidney functional assays respectively, and data were analyzed using SPSS version 20.

Results: FRFFS significantly ($p < 0.05$) increased pack cell volume, hemoglobin, red blood cells and platelets, and significantly decreased white blood cells, most especially at 120 mg/kg and 60 mg/kg doses. Similarly, it significantly increased kidney urea and creatinine concentrations.

Conclusion: Sequel to these findings, it is inferred that FRFFS could be a potential natural therapy for hematological and renal disorders.

Keywords: Flavonoid-rich fraction; Hematopoietic; Inflammation; Metabolites; Renal disorders

1. Introduction

Hematological and renal disorders are fast becoming epidemic. Habib *et al*. [1] labeled chronic kidney disease (CRD) as a global public health problem that could lead to numerous hematological and biochemical dysfunction due to progressive deterioration in kidney structure and function, which further make patients vulnerable to cardiovascular morbidity and mortality if appropriate measures are not taken for its control. Hematology, which is the study of blood and blood disorders, considers problems associated with red blood cells (RBCs), white blood cells (WBCs), platelets (Plts), blood vessels, bone marrow, lymph nodes, spleen and the proteins involved in bleeding and cutting (hemostasis and thrombosis) [2, 3]. During infections or diseased conditions like inflammation, hematopoietic system is seriously affected, and this in turn affects blood cell formation, myeloid and lymphoid cell lines, as well as plasma components of the blood [4, 3]. Inflammation could also affect kidney's structure and metabolites' concentration, and on the long run, its overall functionality. Moreover, hematological disorders such as anemia, leukopenia, neutropenia, leukocytosis, thrombocytosis, thrombocytopenia, monocytosis, lymphocytosis, hemoglobinemia and hemoglobinuria etc are

* Corresponding author: Sunday Adeola Emaleku
Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

associated with inflammatory diseases [5, 6, 7]. Thus, there is a link between inflammation, hematological disorder and kidney functionality. Kidney diseases are associated with changes in many biochemical and hematological parameters [1]. Any observed hematological abnormality in blood and/or deviation in concentration of kidney's metabolites in serum and/or kidney will be valuable aids to diagnose diseased conditions in the blood and/or kidney respectively [3], and would often be useful indicators for providing clue to diagnosis, assessing prognosis and indicating complication of underlying infection, as well as response to therapy [2, 6]. So, it is the search for solution to some of the health challenges faced by man that led to the discovery of *Ficus sur* Forssk. 1775 (Moraceae), a plant commonly known as Bush fig. It is being used as folklore in traditional medicine in many parts of Africa; and Emaleku *et al*. [8] previously reported its hypolipidemic potential in Wistar rats. This study therefore evaluates the effects of FRFFS on hematological parameters and renal metabolites (kidney functional indices) in acetic acid-induced inflammatory Wistar rats.

2. Material and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Acetic acid and n-Hexane were obtained from Sigma-Aldrich, USA; Trizol reagent was obtained from Zymo Research, USA; DNAse was obtained from Thermo Fisher Scientific, USA; while low electroendosmosis (LE) agarose gel, nuclease free water and mix buffer and Tris-borate-EDTA (TBE) buffer were obtained from Inqaba Biotech., West Africa Ltd, and high-performance liquid chromatography (HPLC) grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Others like HPLC grade formic acid and HPLC grade water (18 MX was prepared using a Millipore Milli-Q purification system) were obtained from Acros Organics (Fair Lawn, NJ, USA) and Millipore Corporation, Bedford, MA, USA respectively. Urea and creatinine randox kits were obtained from Randox Laboratories, UK.

2.2. Collection and Authentication of Plant

Ficus sur (FS) leaf was gotten from Iwaro-Oka bush in Akoko area of Ondo State. It was identified and authenticated by Oluwafemi Omotayo of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State. The plant specimen was deposited in Ekiti State University, Ado-Ekiti, Ekiti State Herbarium and assigned Voucher Number: UHAE2018033.

2.3. Plant Preparation

The protocols for the extraction of FRFFS are shown in Fig. 1.

2.4. Extraction and Solvent-Solvent Partitioning

The extraction procedures and solvent-solvent partitioning techniques used in this study were done according to Agbo *et al*. [9] protocols with slight modifications. Fresh leaf (4.0 kg) of FS was washed with water to remove dirt, air dried for three weeks and pulverized into powder using electric blender. The powdered leaf (1.3 kg) was macerated with 3.5 L of methanol at room temperature for 72 h and filtered with Whatman number 1 filter paper. It was then concentrated using rotary evaporator at 45 ^{0}C. The crude extract (methanolic fraction) was purified using partitioning technique with water and n-hexane at 2:1 v/v (to obtain 85.2 g and 126.4 g yield respectively) and then water and ethyl acetate (EA) at 1:1 v/v (to obtain 39.1 g and 41.1 g yield respectively) successively. The resulting fractions; aqueous fraction and ethyl acetate (EA) fraction were freeze-dried using freeze dryer. The EA fraction was used for animal study.

2.5. Secondary Metabolites Screening of *Ficus sur* (FS)

Secondary metabolites i.e. bioactive compounds present in methanolic extract of FS plant were qualitatively carried out using standard procedures according to Sofowora [10] protocols, which is colorimetric-based.

2.6. Animals Used for the Study

Ethical approval was obtained from Research and Development Center of Adekunle Ajasin University, Akungba-Akoko, Ondo State. Healthy male Wistar rats, average weight of 112.60 ± 0.75 g were purchased from the Animal House, Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH), Ibadan. The animal were acclimatized for two weeks and maintained in line with National Institute of Health [11] guide for the care and use of laboratory animals. They were kept in a wooden cage housed in the Animal House of the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State and fed standard pellets feed and water *ad libitum* at room temperature of 22 ± 2 ^{0}C and 55 ± 5% relative humidity where equal period (hour) of light and darkness were maintained (i.e. 12 h light/12 h dark).

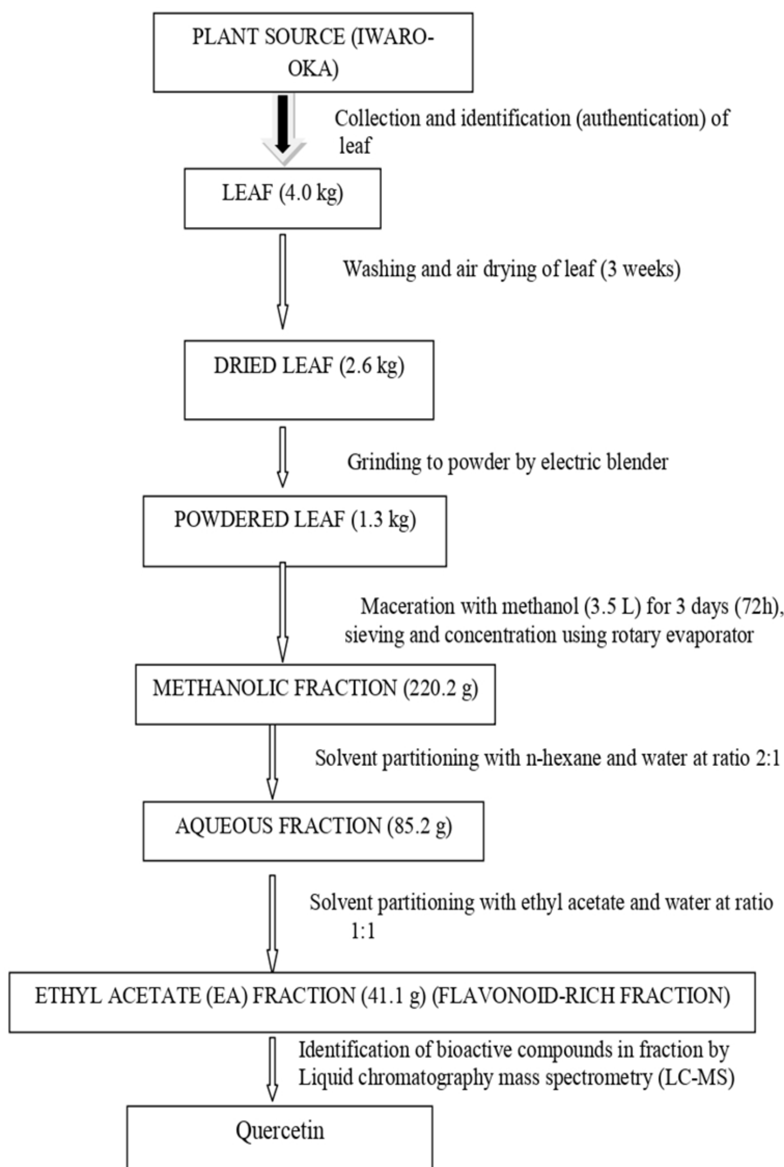


Figure 1 Flow chart of preparation of Flavonoid-rich Fraction of *Ficus sur*

2.7. Inflammation Induction Process

A modified method of Tannahill *et al*. [12] was used to induce inflammation in the animals (except normal control (NC) group) by successive oral administration of 6% at (20 ml/kg), 3% at (15 ml/kg) and 6% at (10 ml/kg) acetic acid within nine (9) days. A 20 ml/kg of 6% acetic acid was first administered to the animals. Three days after, the dosage and concentration were reduced to 15 ml/kg and 3% respectively i.e. 15 ml/kg of 3% acetic acid, and administered twice to the animals at two days interval. Finally, 10 ml/kg of 6% acetic acid was administered to the animals for two consecutive days. The administration was done once a day in the morning, around 7.30 am. The inflamed animals were used for this study.

2.8. Experimental Design

Thirty-five (35) Wistar rats comprising of thirty (30) inflamed rats and five (5) non-inflamed rats were randomly divided into six (6) groups of five animals each (n = 5) that formed group II to VII and normal control group (i.e. group I) respectively as shown below:

Group I: Normal Control (NC) rats, orally administered 1 ml of distilled water

Group II: Positive Control (PC) rats, orally administered 15 mg/kg Celecoxib
Group III: Negative Control (NegC) rats, orally administered 1 ml of distilled water
Group IV: FRFFS group (rats), orally administered 15 mg/kg of FRFFS
Group V: FRFFS group (rats), orally administered 30 mg/kg of FRFFS
Group VI: FRFFS group (rats), orally administered 60 mg/kg of FRFFS
Group VII: FRFFS group (rats), orally administered 120 mg/kg of FRFFS

Treatments were done once a day in the morning and lasted for fourteen (14) days. At the end of treatments, experimental animals were sacrificed via cardiac puncture under mild anesthesia using chloroform after an overnight fast. Blood and kidney samples were collected for full blood count (FBC) and renal metabolites assays respectively.

2.9. Full Blood Count/Hematological Parameters Analysis

Hematological parameters such as WBCs count with differentials (Lym, Mid, Gran), hemoglobin (Hgb) level, RBC count, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), Plt and mean platelet volume (MPV) were determined by automation means, using hematological analyzer (URIT 3300 Automated Hematology Analyzer, URIT Medical Electronic Co. Ltd., China).

2.10. Renal Metabolites Assay

2.10.1. Determination of Urea and Creatinine

Urea was determined by following instructions on the randox kit manual (Urease-Berthelot Method) in accordance with Machado and Horizonte [13] method, while creatinine was determined according to Haeckel [14] method.

2.11. Gene Expression Protocol

Reverse transcriptase polymerase chain reaction (RT-PCR) protocol was used to determine the relative gene expression of pro-inflammatory mediators; tumor necrosis factor-α (TNF-α) and interleukin 12 (IL-12) according to Burgmann *et al*. [15] protocol with slight modifications.

2.11.1. RNA Isolation

RNA was isolated with Trizol reagent from liver tissue. DNA contaminant was removed using DNAse I treatment by following the manufacturer's instructions. The isolated RNA was reconstituted into nuclease free water and quantified by measuring the absorbance at 260 nm using Hitachi-U1900 spectrophotometer.

2.11.2. cDNA Synthesis

Isolated RNA was reversely transcribed (reverse transcription reaction) to complementary deoxyribonucleic acid (cDNA) using Proto Script ll First Strand cDNA synthesis kit (BioLabs, New England) in a 3-step reaction condition: 65 ^{0}C for 5 min, 42 ^{0}C for 1 hr and 70 ^{0}C for 5 min.

2.11.3. PCR Amplification

This was done using One-Taq 2X Master Mix (BioLabs, New England). The reaction was run on a Labgene thermocycler in an amplification conditions of 94 ^{0}C pre-denaturation for 5 min, denaturation at 94 ^{0}C for 30 sec, annealing at 55 ^{0}C (Tm) for 30 sec and extension at 72 ^{0}C for 30 sec and then, 5 min at 72 ^{0}C for further and final extension in 30 cycles. Primers used were purchased from Inqaba Biotech., West Africa Ltd (Hatfeild, SA) and are listed in Table 1. The amplicons were electrophoresed in 0.5% agarose gel using electrophoresis machine (Ebman Biomedical).

Table 1 Primers of the selected pro-inflammatory mediators

S/N	Gene	Forward Primer Sequence (5′ – 3′)	Reverse Primer Sequence (5′ – 3′)
1	TNF-α	ACCACGCTCTTCTGTCTACTG	CTTGGTGGTTTGCTACGAC
2	IL-12	GCTCTGGCTTAGGTGTGATT	CCGTCTCCGTATCTGGATTTC
3	GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

2.12. Statistical Analysis of Data

Intensities of bands formed on agarose gel electrophoresis were quantified densitometrically using Image J software and the gene expression was normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, a housekeeping gene. All data collected were subjected to one-way analysis of variance (ANOVA) and differences in means between groups were determined by Tukey post hoc test i.e. Tukey's Krammer Least significant difference (LSD) test using SPSS version 20. The results were expressed as mean ± standard error of mean (mean ± SEM), and values of $p < 0.05$ (i.e., 95% Confidence Level) were considered statistically significant.

3. Results

3.1. Secondary Metabolites

Flavonoids, phenols, cardiac glycosides, tannins, phlabotannins, saponins, terpenoids, phytates, alkaloids and oxalates secondary metabolites are present in FS plant (see Table 2).

Table 2 Secondary metabolites screening of *Ficus sur* plant

Secondary Metabolites	(+) or (-)
Tannins	+
Phlabotannins	+
Saponins	+
Terpenoids	++
Flavonoids	++
Phenols	+
Phytates	+
Alkaloids	+
Oxalates	+
Steroids	-
Cardiac glycosides	+

The signs (+) and (–) denote present and absent respectively

3.2. Hematological Parameters

There is significant ($p < 0.05$) increase in PCV, Hgb, RBC and Plt concentrations in FRFFS-treated groups, most especially at 120 mg/kg and 60 mg/kg when compared with NegC and/or PC group(s). Conversely, FRFFS significantly decreases WBC and Lymphocytes (Lym) when compared with NegC and PC groups (see Table 3).

Table 3 Effect of flavonoid-rich fraction of *Ficus sur* on hematological parameters of animals

Assay	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
PCV (%)	51.15±0.40^{b}	48.30±0.46^{c}	47.03±0.40^{cd}	48.70±0.48^{bc}	49.10±0.27^{bc}	52.69±0.50^{ab}	55.10±0.56^{a}
Hgb (g/dl)	13.45±0.20^{ab}	13.22±0.64^{ab}	11.22±0.35^{c}	13.53±0.40^{ab}	13.50±0.21^{ab}	13.42±0.32^{ab}	14.35±0.25^{a}
RBC ($\times10^6$/µl)	6.68±0.21^{ab}	7.25±0.12^{a}	6.38±0.35^{b}	6.88±0.11^{ab}	7.10±0.72^{ab}	7.33±0.11^{a}	7.39±0.17^{a}
MCV (fl)	70.30±0.73^{ab}	69.80±0.79^{ab}	69.57±0.61^{ab}	72.27±0.43^{a}	72.40±0.40^{a}	72.10±0.90^{a}	72.77±0.50^{a}
MCH (pg)	19.84±0.50^{a}	19.78±0.44^{a}	19.13±0.10^{ab}	19.05±0.26^{ab}	19.97±0.44^{a}	20.80±0.59^{a}	19.63±0.12^{a}
MCHC (%)	27.48±0.40^{a}	26.98±0.72^{ab}	26.15±0.61^{ab}	27.66±0.17^{a}	27.63±0.22^{a}	27.98±0.45^{a}	27.28±0.56^{a}
WBC ($\times10^3$/µl)	7.27±0.70^{c}	10.13±0.58^{a}	9.13±0.45^{ab}	6.13±0.35^{d}	7.15±0.45^{c}	6.73±0.66^{d}	7.80±0.74^{c}
Lym ($\times10^3$/µl)	5.02±0.56^{c}	8.13±0.44^{a}	8.79±0.52^{a}	5.14±0.33^{c}	6.05±0.32^{b}	4.90±0.22^{c}	6.15±0.53^{b}
Mid ($\times10^3$/µl)	0.53±0.33^{d}	0.93±0.03^{ab}	1.04±0.04^{a}	0.52±0.03^{d}	0.67±0.03^{c}	0.63±0.03^{c}	0.90±0.00^{ab}
Gran ($\times10^3$/µl)	1.05±0.60^{c}	1.67±0.02^{a}	1.30±0.01^{b}	1.45±0.25^{ab}	1.33±0.33^{b}	1.10±0.04^{c}	1.45±0.09^{ab}
Plt ($\times10^3$/µl)	646.00±2.52^{ab}	641.00±2.52^{b}	549.33±3.38^{c}	637.33±1.20^{b}	643.33±3.00^{ab}	644.50±2.72^{ab}	656.67±3.18^{a}
MPV (fl)	8.33±0.22^{a}	8.30±0.09^{a}	6.98±0.10^{b}	8.18±0.17^{ab}	8.27±0.17^{ab}	8.52±0.45^{a}	8.20±0.27^{a}
PCT (%)	0.55±0.01^{a}	0.53±0.01^{a}	0.41±0.01^{b}	0.55±0.01^{a}	0.57±0.02^{a}	0.55±0.01^{a}	0.55±0.02^{a}
PDW (fl)	9.25±0.17^{a}	8.98±0.13^{ab}	8.60±0.39^{b}	9.23±0.14^{ab}	9.03±0.09^{ab}	9.54±0.39^{a}	9.33±0.15^{ab}

Keys: **NC** – Normal Control; **PC** – Positive Control; **Cxb** – Celecoxib; **NegC** – Negative Control; **FREFS** – Flavonoid-Rich Fraction of *Ficus sur*; **PCV** – Packed Cell Volume; **Hgb** – Haemoglobin; **RBC** – Red Blood Cell; **MCV** – Mean Corpuscular Volume; **MCH** – Mean Corpuscular Hemoglobin; **MCHC** - Mean Corpuscular Hemoglobin Concentration; **WBC** – White Blood Cell; **Lym** – Lymphocyte; **Mid** – Monocyte; **Gran** – Granulocyte; **Plt** – Platelet; **MPV** – Mean Platelet Volume; **PCT** – Plateletcrit; **PDW** – Platelet Distribution Width; **Group 1** – NC; **Group 2** - PC (15 mg/kg Cxb); **Group 3** – NegC; **Group 4** - 15 mg/kg FRFFS; **Group 5** - 30 mg/kg FRFFS; **Group 6** - 60 mg/kg FRFFS; **Group 7** - 120 mg/kg FRFFS.

Note: comparison is strictly between groups (within parameter), and Means ± Standard Error of Means (SEMs) with the same superscript(s) do not differ significantly from each other. Statistical significance was considered at $p < 0.05$.

3.3. Renal Metabolites

There is significant ($p < 0.05$) increase in kidney urea in 120 mg/kg and 60 mg/kg FRFFS groups when compared with NegC group. Likewise, there is significant increase in kidney creatinine in 120 mg/kg and 60 mg/kg FRFFS-treated groups when compared with NegC and PC groups. Also, the kidney urea and creatinine of both NC and PC groups are significantly different from that of NegC group (see Table 4).

Table 4 Effect of flavonoid-rich fraction of *Ficus sur* on kidney urea and creatinine of animals

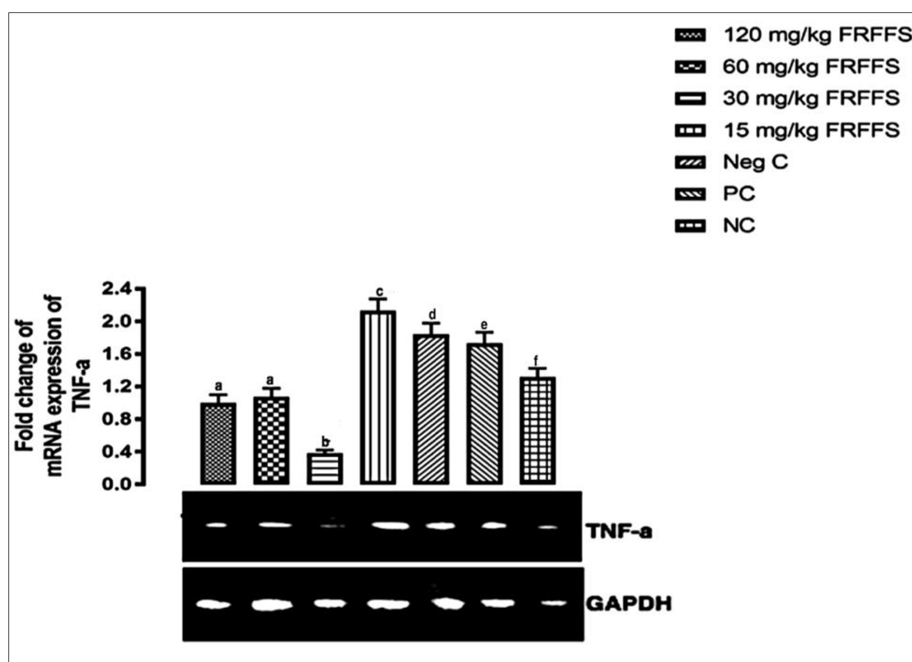
Assay	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Urea(mg/dl)	63.75±1.16[ab]	64.23±1.01[ab]	55.34±1.10[c]	60.44±0.93[abc]	60.35±2.49[abc]	69.28±1.78[a]	67.99±1.86[a]
Creat (mg/dl)	2.29±0.06[bc]	1.88±0.04[c]	1.39±0.06[d]	2.02±0.50[bc]	2.25±0.12[bc]	2.61±0.28[a]	2.59±0.11[a]

Keys: Creat – Creatinine; Group 1 – NC; Group 2 - PC (15 mg/kg Cxb); Group 3 – NegC; Group 4 - 15 mg/kg FRFFS; Group 5 - 30 mg/kg FRFFS; Group 6 - 60 mg/kg FRFFS; Group 7 - 120 mg/kg FRFFS

Note: comparison is strictly between groups (within parameter), and Means ± Standard Error of Means (SEMs) with the same superscript(s) do not differ significantly from each other. Statistical significance was considered at $p < 0.05$.

3.4. Gene Expression of Pro-inflammatory Mediators

There is significant ($p < 0.05$) down regulation of TNF-α gene in 120 mg/kg, 60 mg/kg and 30 mg/kg FRFFS groups when compared with NegC, PC and NC groups as shown in Fig. 2.



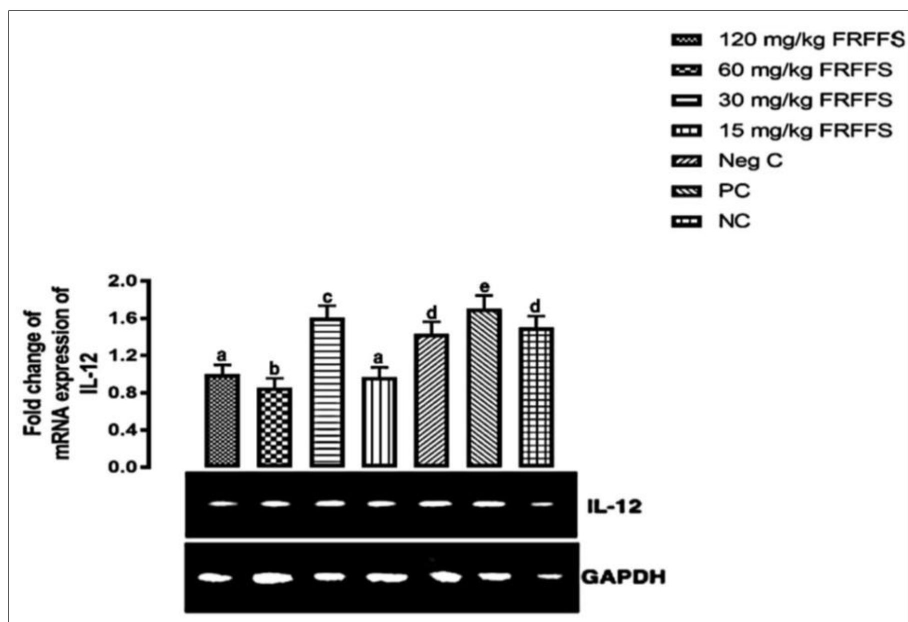
Note: The keys at the top right corner show that this bar chart grouping is from 120 mg/kg to NC i.e. left to right respectively.

Key: mRNA – messenger ribonucleic acid; TNF-a – tumor necrosis factor-alpha; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; FRFFS – flavonoid-rich fraction of Ficus sur; NegC – negative control; PC – positive control; NC – normal control.

Figure 2 TNF-alpha gene expression of experimental animals

The values are presented as mean ± SEM (n = 5). Groups with different alphabets are significantly different from one another at $p<0.05$.

Similarly, IL-12 gene is significantly ($p < 0.05$) down regulated especially in 120 mg/kg and 60 mg/kg FRFFS groups when compared with NegC, PC and NC groups as shown in Fig. 3.



Note: The keys at the top right corner show that this bar chart grouping is from 120 mg/kg to NC i.e., left to right respectively.
Key: mRNA – messenger ribonucleic acid; IL-12 – interleukin 12; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; FRFFS – flavonoid-rich fraction of Ficus sur; NegC – negative control; PC – positive control; NC – normal control

Figure 3 IL-12 gene expression of experimental animals

The values are presented as mean ± SEM (n = 5). Groups with different alphabets are significantly different from one another at p<0.05.

4. Discussion

4.1. Effects of Flavonoid-Rich Fraction of *Ficus Sur* on Hematological Parameters and Renal Metabolites

Keeping the blood and kidney (an important organ used for excretion and osmoregulation) perpetually healthy is very essential for their functional roles and the general wellbeing of the body. Their healthiness helps maintain their structural and biochemical integrities that in turn determine the overall functions of the body. Thus, the observed significantly higher PCV, Hgb, RBC and Plt concentrations in FRFFS-treated groups, most especially at 120 mg/kg and 60 mg/kg dosages than NegC group and/or PC group (see Table 3) suggests the ability of FRFFS to maintain these hematological parameters' levels and prevents blood disorders, since their concentrations are not significantly different from that of the control group, NC. It could further be adduced that FRFFS has inherent potentials to prevent and/or treat hematological disorders like anemia, hemoglobinemia, hemoglobinuria hemolysis, thrombocytopenia, hemorrhage, blood dehydration and other blood disorders or complications. According to Enawgaw *et al*. [6] and Abay *et al*. [3], these hematological parameters are valuable aids to diagnose many hematological abnormalities and diseased conditions since they serve as useful indicators that provide clue to diagnosis, assessing prognosis and indicating complication of underlying infection, as well as response to therapy [2]. Hence, the finding of this study places FRFFS as a natural therapy to anemia and other hematological disorders, as well as, many other metabolic diseases because; adequate hemoglobin concentration is needed for oxygen transportation throughout the body and in many metabolic processes like electron transport or respiratory chain, enzyme catalysis that involves cytochrome P-450 enzymes, cycloogynases etc. Moreover, enough RBCs must be present in the body in order to have adequate quantity of hemoglobin required for oxygen transportation [7]; and both parameters are of adequate concentrations in FRFFS-treated groups (see Table 3).

Conversely, the significantly low concentrations of PCV, Hgb, RBC and Plt concentrations, and significantly high WBC counts observed in NegC group as seen in Table 3 indicate negative alterations in hematological parameters in the group that can be very devastating. It is an indicative sign of anemia, thrombocytopenia, hemorrhage, as well as, compromised immunity that could lead to unresponsiveness of immune system cells during pathogenic invasion, infectious disease or any chemical attack to the body. For instance, it is already known that anemic condition as a result of low RBC would result to deficient glucose-6-phosphate (G6PD) activity that would in turn lead to depleted NADPH and consequently, oxidative damage [16]. Therefore, all the abnormal changes in hematological parameters of NegC group (see Table 3)

could be linked with many other diseased conditions. According to Abay *et al*. (2018), many hematological abnormalities in terms of parameters are observed during diseased conditions that include kidney diseases. Kidney diseases are associated with changes in various biochemical and hematological parameters [1]. Anemia is parallel to the degree of renal impairment, mostly caused by failed secretion of renal erythropoietin [17, 18, 19, 1]. In fact, Habib *et al*. [1] stated that "the essential cause of decreased RBC counts and consequent decrease in Hgb and PCV concentrations in chronic renal failure is impaired erythropoietin production and other factors, which suppress marrow erythropoiesis and shortened red cell survival". It is therefore logical to attribute the abnormalities in these hematological parameters observed in NegC group to renal/kidney malfunctioning (among other factors), which the significantly low kidney urea and creatinine concentrations (see Table 4) in the group seems to affirm. To corroborate this, Means and Glader [20] and recently, Habib *et al*. [1] reported that RBC survival in uremic patients decreased proportionally to blood urea nitrogen concentration; and it will interest you to know that both RBC and kidney urea are decreased in this group but, increased significantly in FRFFS-treated groups, especially at 120 mg/kg and 60 mg/kg dosages in this study, which suggests FRFFS' ability to ameliorate renal dysfunction or disorder. So, the appropriate concentrations of these hematological parameters, as well as, high kidney urea and creatinine concentrations of FRFFS-treated groups suggests absence of hematological and renal disorders in the animals, and underscores its usefulness as potential natural therapy for both diseased conditions. According to Higgins [21] and Gounden and Jialal [22], increased kidney creatinine and urea concentrations as observed in FRFFS groups implies that glomerular filtrate rate (GFR) of the kidney is functional and is disease-free.

Ficus sur (FS), formerly known as *Ficus capensis* has been reported by Daikwo *et al*. [23] and Esievo *et al*. [24] to possess pharmacological properties such as immune booster, wound/sore-healing, and antioxidant among others. It can then be adduced that FRFFS possesses pharmacological or therapeutic potentials for hematological disorders and renal diseases due to the wide arrays of secondary metabolites such as flavonoids, phenols, cardiac glycosides, tannins, saponins, terpenoids, alkaloids etc found in it in this study (see Table 2). Ramde-tiendrebeogo *et al*. [25] also found most of these secondary metabolites in the leaf of FS. More recently, Saloufou *et al*. [26] reported the presence of alkaloids, flavonoids, glycosides, tannins, terpenoids, steroids and anthocyanins in the leaf of FS. According to Altemimi *et al*. [27], plants' phenolic compounds such as flavonoids and tannins in addition to their analgesic, anti-inflammatory, anti-mutagenic and anxiolytic properties, possess antioxidant property that could protect against free radical damage when consumed. They help the body to scavenge free radicals as natural antioxidants and thus, keep the body integrity intact.

It is important to note that celecoxib, the non-steroidal anti-inflammatory drug (NSAID) used as positive control in this study did not cause any significant negative alteration in hematological parameters and renal metabolites against some contrary literatures reports that NSAIDs cause kidney failure when compared with the NC group.

4.2. Effects of Flavonoid-Rich Fraction of *Ficus sur* on Pro-inflammatory Genes

The up regulation and down regulation of pro-inflammatory genes in NegC and/or PC group(s) and FRFFS-treated groups respectively as observed in Fig. 2 and 3 imply that acetic acid truly induced inflammation in the Wistar rats via its oral administration and FRFFS (especially at the dosages of 120 mg/kg and 60 mg/kg) worked therapeutically to ameliorate the induced inflammatory condition in FRFFS-treated animals (i.e. anti-inflammatory effect) respectively. The increased WBC in NegC group is opined to be the reason for the significant up regulation of TNF-α and IL-12 genes as observed in Fig. 2 and 3 because they are positively correlated. According to Singh and Goyal [28], these pro-inflammatory mediators are produced by reactive thrombocytosis and increased thrombopoietic by inflamed cells as a result of inflammatory response. This result is in accordance with Hagar *et al*. [29] and Yalniz *et al*. [30] findings, who previously reported the use of acetic acid in the induction of inflammatory diseases like inflammatory bowel disease and colitis in experimental animals. Fig. 2 and 3 results consequently affirm acetic acid's potency to induce inflammation and equally serves as testament that the experimental animals were truly inflamed i.e. inflammation-induced. The findings from Fig. 2 and 3 results further suggest that FRFFS could be used to assuage inflammation as it down regulated TNF-α and IL-12 expression levels in the animals; and this anti-inflammatory property is also credited to the various secondary metabolites found in the plant.

5. Conclusion

Since evaluation of hematological parameters is a valuable means of assessing blood composition/component and functionality in relation to diseases; and based on FRFFS' ability to maintain and/or improve indicators (parameters) of both hematological and renal disorders, it is therefore inferred that FRFFS, most especially at 120 mg/kg and 60 mg/kg dosages would be a potential natural therapy for both diseased conditions.

Compliance with ethical standards

Acknowledgments

I appreciate the technical support of Bioinformatics and Molecular Biology Unit's staff of the Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria during this study. My profound gratitude goes to Mr. Joshua Logo and late Mr. Francis Adeyemi for providing the *Ficus sur* (FS) sample used for this study. The supports, motivations and encouragements from my family members during this study are highly recognized; and I will not forget to thank my research team for their supports always.

Disclosure of conflict of interest

I declare no conflict of interest in this study.

Statement of ethical approval

Ethical consideration was duly observed by the author.

References

[1] Habib A, Ahmad R, Rehman S. Hematological changes in patients of chronic renal failure and the effect of hemodialysis on these parameters. Int. J. Res. Med. Sci. 2017; 5(11): 4998-5003.

[2] Dikshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of hematological abnormalities of Indian HIV infected individuals. BMC Blood Disorders. 2009; 9(5): 234-242.

[3] Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological abnormalities of pulmonary tuberculosis patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: a comparative cross-sectional study. Tuberculosis Research and Treatment. 2018; 1-7.

[4] Bakhubaira S. Hematological parameters in severe complicated Plasmodium falciparum malaria among adults in Aden. Turk. J. Haematol. 2013; 30: 394-399.

[5] Rohini K, Bhat MS, Srikumar PS, Kumar AM. Assessment of hematological parameters in pulmonary tuberculosis patients. Indian Journal of Clinical Biochemistry. 2016; 31(3): 332-335.

[6] Enawgaw B, Adane N, Terefe B, Asrie F, Melku M. A comparative cross-sectional study of some hematological parameters of hypertensive and normotensive individuals at the University of Gondar hospital, Northwest Ethiopia. BMC Hematol. 2017; 17: 21-27.

[7] Wikipedia. Hematology. © 2019 [cited 2019 October 2].

[8] Emaleku SA, Adanlawo IG, Gbadegesin MT, Adeola EA, Omueti OD, Emaleku GO, Adeyemi AF, Ogunbodede AD. Determination of lipid profile in inflammation-induced Wistar rats administered Ficus sur flavonoid-rich extract. Innovation and Advancement in Nanoscience and Nanotechnology. Proceedings of the 2019 International Science Conference, Federal University Oye-Ekiti, 8th – 10th July, 2019; 43-47.

[9] Agbo MO, Lai D, Okoye FBC, Osadebe OP, Proksch P. Antioxidative polyphenols from Nigerian mistletoe Loranthus micranthus (Linn.) parasitizing on Hevea brasiliensis. Fitoterapia. 2013; 86: 78-83.

[10] Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria. 1993; 289-356.

[11] National Institutes of Health. Guide for the care and use of laboratory animals. National Research Council (US) Committee for the update of the guide for the care and use of laboratory animals. National Academy of Sciences. 8th Edition. Washington (DC): National Academies Press (US). 2011.

[12] Tannahill CL, Stevenot SA, Campbell-Thompson M, Nick HS, Valentine JF. Induction and immunolocalization of manganese superoxide dismutase in acute acetic acid-induced colitis in the rat. Gastroenterology. 1995; 109: 800-811.

[13] Machado M, Horizonte B. Simple and rapid method for determination of urea by urease. Rev. Assoc. Med. Bras. 1958; 4: 364-367.

[14] Haeckel R. Assay of creatinine in serum, with use of fuller's earth to remove interferents. Clin. Chem. 1981; 27: 179-183.

[15] Burgmann H, Burgmann G, Widmer F, Sigler WV, Zeyer J. mRNA extraction and reverse transcription-PCR protocol for detection of nifH gene expression by Azotobacter vinelandii in Soil. Applied and Envr. Microbiol. 2003; 69(4): 1928-1935.

[16] Wahed MDA, Dasgupta A. Red blood cell disorders. Hematology and Coagulation. 2015; 3(4): 2-8.

[17] Hsu CY, Bates DW, Kuperman GJ, Curhan GC. Relationship between hematocrit and renal function in men and women. Kidney International. 2001; 59(2): 725-731.

[18] Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. Kidney International. 2007; 71(1): 31-38.

[19] Locatelli F, Pozzoni P, Del Vecchio L. Recombinant human epoietin beta in the treatment of renal anaemia. Ther. Clin. Risk Manag. 2007; 3(3): 433-439.

[20] Means RT, Glader B. Acquired nonimmune hemolytic disorders. Wintrobe's Clinical Hematology. 2009; 1: 1021-1037.

[21] Higgins C. Urea and creatinine concentration, the urea-creatinine ratio. © 2016 [cited 2019 October 25].

[22] Gounden V, Jialal I. Renal function tests. Creative Commons Attribution 4.0 International License. © 2019 [cited 2019 October 25].

[23] Daikwo OA, Tende JA, Okey SM, Eze ED, Isa AS. The effect of aqueous extract of leaf of Ficus capensis Thunb (moraceae) on in vivo leukocyte mobilization in Wistar rats. Bri. J. Pharmacol. Toxicol. 2012; 3(3): 110-114.

[24] Esievo BE, Anthony SO, Fatokun OT, Kunle OF. Ficus capensis Thumb. (moraceae): review of its ethnomedicinal uses, pharmacological activities and phytochemical constituents. Archives of Current Research International. 2018; 12(3): 1-7.

[25] Ramde-Tiendrebeogo A, Tibiri A, Hilou A, Lompo M, Millogo-Kone H, Nacoulma OG, Guissou IP. Antioxidative and antibacterial activities of phenolic compounds from Ficus sur Forssk and Ficus sycomorus L. (moraceae): potential for sickle cell disease treatment in Burkina Faso. Int. J. Biol. Chem. Sci. 2012; 6(1): 328-336.

[26] Saloufou KI, Boyode P, Simalou O, Eloh K, Idoh K, Melila M, Outéndé Toundou O, Kpegba K, Agbonon A. Chemical composition and antioxidant activities of different parts of Ficus sur. J. Herb. Med. Pharmacol. 2018; 7(3): 185-192.

[27] Altemimi A, Lakhssassi N, Baharlouei A, Watson AD, Lightfoot DA. Phytochemicals: extraction, isolation and identification of bioactive compounds from plant extracts. Plants. 2017; 6(4): 1-23.

[28] Singh PP, Goyal A. Interleukin-6: a potent biomarker of mycobacterial infection. Springer Plus. 2013; 2(686): 123-134.

[29] Hagar HH, El-Medany A, El-Eter E, Arafa M. Ameliorative effect of pyrrolidine dithiocarbamate on acetic acid-induced colitis in rats. Eur. J. Pharmacol. 2007; 554: 69-77.

[30] Yalniz M, Demirel U, Orhan C, Bahcecioglu IH, Ozercan IH, Aygun C, Tuzcu CM, Sahin K. Nadroparin sodium activates Nrf2/HO-1 pathway in acetic acid-induced colitis in rats. Inflammation. 2012; 35: 1213-1221.