



## Synergistic Pharmacological Effect of Leaf Extracts of *Ficus exasperata* and *Telfeira occidentalis* on Chloramphenicol - Induced Anaemia in Wistar Rats

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### Authors' contributions

This work was carried out in collaboration among all authors. Author NAPC designed the study. Author AEAA performed the statistical analysis. Author IPA wrote the protocol and the first draft of the manuscript. Author AUM managed the analyses of the study. Author URI managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Objective:** This study was undertaken to validate the haematinic potentials of the aqueous leaf extracts of *Ficus exasperata* (FEAE) and *Telfeira occidentalis* (TOAE) administered separately and to establish a possible synergistic interaction when administered concurrently to chloramphenicol-induced anaemic rats.

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**Materials and Methods:** Anaemia was induced by oral administration of chloramphenicol (50 mg/kg) for 2 weeks. Five (5) non- anaemic rats and Twenty (20) anaemic rats were used in this study. The non – anaemic rats served as non- anaemic control and received 1ml dist. H<sub>2</sub>O. The 25 anaemic rats were divided into 5 groups of 5 rats each (groups 2- 6). Group 2 served as anaemic control and received 1 ml dist. H<sub>2</sub>O, group 3 served as positive control (reference drug) and received ferrous gluconate (900 mg/kg) while groups 4- 6 received FEAE (200 mg/kg), TOAE (200 mg/kg) and FEAE (100 mg/kg) + TOAE (100 mg/kg) respectively. Treatment was carried out once daily for 7 days after which the rats were bled for determination of PCV, Hb and WBC count.

**Results:** Chloramphenicol induced a significant decrease PVC and Hb indicating anaemia and also resulted to a significant increase in WBC count. Aqueous leaves extracts of *Ficus exasperata* and *Telfeiria occidentalis* produced significant increase in PCV and Hb with a corresponding decrease in WBC after 7 days of oral administration to anaemic rats. The anti- anaemic effect observed with co-administration of the extracts was however, significantly more than either of the extract administered alone.

**Conclusion:** It can be concluded that the extracts of *Ficus exasperata* and *Telfeiria occidentalis* show synergistic effect when co-administered. This could be useful in the management of anaemia.

**Keywords:** Synergistic; pharmacological; *Telfeiria occidentalis*; chloramphenicol; anaemia.

## 1. INTRODUCTION

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections [1]. In anaemia there is decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in females [2]. In the tropics, due to endemicity of malaria, between 10 to 20% of the population presents less than 10 g/dl of Hb [3]. Children are more vulnerable.

A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Telfeiria occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavenscens* and *Brillantasia nitens*, *Ficus exasperata* [4,5]. This study was carried out to validate the haematinic effect of *Telfeiria occidentalis* and *Ficus exasperata* when administered to rats separately and also to establish a possible synergistic effect when the two are administered concurrently.

*Ficus exasperata* Vahl (Moraceae) is an important medicinal plant with a wide geographical distribution in Africa particularly in Nigeria [6,7]. The leaves are oval, it has elliptic leaves with a very rough surface and are alternately arranged making them look like sand paper. *Ficus exasperata* (Vahl) is commonly known as sand paper tree (“*Ewe Ipin*”) in Yoruba. Other various local names include; *Anwerenwa* (Igbo), *Erepin* (Yoruba), *Kawusa* (Nupe), *Ameme* (Edo) [8]. The leaf extract from *Ficus exasperata*

is reported to have diverse uses such as treating hypertensive patients [9], coughs and haemorrhoid [10]. Numerous pharmacological actions such as lipid lowering, anti-diabetic and antifungal activities have been reported for *Ficus exasperata* [11], and more recently the use of *Ficus exasperata* for treating several problems like difficult child birth, bleeding and diarrhoea in traditional medicine was reported [12].

*Telfeiria occidentalis* known as fluted pumpkin occurs in the forest zone of West and Central Africa; they are found more in Benin, Nigeria and Cameroon [13]. It is a well-known vegetable all over Nigeria. It was found first in South-east Nigeria and was distributed by the Igbos’, who have cultivated this crop for a very long time. It is possible that fluted pumpkin was originally wild throughout its current range, but that wild plants have been harvested to local extinction and are now replaced by cultivation forms [14,15,16]. *Telfeiria occidentalis* has been reported by many investigators to have medicinal attributes. The herbal preparation of the plant has been used in the treatment of anaemia, chronic fatigue and diabetes [17,18]. It is noted that the leaves contain essential oils, vitamins; root contains cucubitacine, sesquiterpene, lactose [19]. In the treatment of convulsion, the young leaves sliced and mixed with coconut water and salt stored in a bottle can be used traditionally [20]. *Telfeiria* leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems [21].

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor. Ferrous gluconate was purchased from a local pharmacy shop.

#### 2.1.2 Animals

Thirty (30) adult Wistar rats of either sex weighing 150–200 g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

### 2.2 Methods

#### 2.2.1 Plant collection and identification

The leaves of *Ficus exasperata* and *Telfeira occidentalis* were collected from a natural habitat in Opi Area of Enugu State, Nigeria. The plants were identified at Pharmacognosy Department, University of Nigeria, Nsukka and voucher specimens were deposited for future references.

#### 2.2.2 Preparation of extracts

The leaves of *Ficus exasperata* and *Telfeira occidentalis* were shade- dried for five (5) days and pulverized separately using an electric blender. One thousand (1000) gram of each of the pulverized leaves was soaked in distilled water separately for 72- hours. The resulting mixtures were filtered using Whatmann filter paper (Size No 1) and the extracts were concentrated using free- dryer. The extracts of *Ficus exasperata* and *Telfeira occidentalis* shall henceforth be referred to as FEAE and TOAE respectively.

#### 2.2.3 Acute toxicity study

The oral median lethal dose (LD<sub>50</sub>) of the extracts was determined in rats according to the method of [22]. The oral median lethal dose (LD<sub>50</sub>) of the extract was determined in rats according to the method described by [15]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given 10, 100,

and 1000 mg extract/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included but were not limited to paw- licking, stretching, respiratory distress and mortality for the first 4 h and thereafter daily for two weeks. Based on the results of the initial phase, the following doses- 1600, 1900 and 5000 mg extract/kg body weight were administered to another set of three groups of three rats in the second phase. These rats were also monitored closely for the first 4 h after treatment and subsequently daily for 14 days for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD<sub>50</sub>.

#### 2.2.4 Induction of anaemia

Haematological parameters were initially determined for untreated rats according to established method [23]. Anaemia was induced by orally administration of chloramphenicol (50 mg/kg) for 2 weeks in four groups and each contains five rats. The Anaemia was confirmed by the markedly low PCV compared to untreated rats.

#### 2.2.5 Anti- anaemic screening

Five (5) non- anaemic rats and Twenty- five (25) anaemic rats were used in this study. The non – anaemic rats served as control and received distilled water. The 25 anaemic rats were divided into 5 groups of 5 rats each (groups 2- 6). Group 2 served as anaemic control, group 3 served as positive control (reference drug) and received ferrous gluconate (900 mg/kg) while groups 4- 6 received 200 mg/kg FEAE, 200 mg/kg TOAE and 100 mg/kg FEAE+ 100mg/kg TOAE respectively. Treatment was carried out once daily for 7 days. All the treated animals were fed with water and pellets for a week. Animals were bled for determination of PCV, Hb, WBC parameters [23], before and after administration of the extracts and reference drug. Percentage recovery was calculated for each haematological parameter, using the following formula typical of PCV:

$$\% \text{ Recovery} = \frac{(\text{PCV post- treatment}) - (\text{PCV pre- treatment})}{(\text{Normal PCV} - \text{PCV pre- treatment})} \times 100$$

#### 2.2.6 Statistical analysis

Data were expressed as mean standard error of mean (SEM). Statistical comparisons were

performed by one-way ANOVA, followed by Tukey- Kramer multiple comparisons test and student-Newman-Keuls multiple comparisons test and the values were considered statistically significant when p-value is less than 0.05 ( $p < 0.05$ )

### 3. RESULTS

#### 3.1 Acute Toxicity

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of aqueous extracts of *Ficus exasperata* and *Telfeira occidentalis*. The oral LD<sub>50</sub> of each of the extracts was then taken to be > 5000 mg/kg.

#### 3.2 Effect of the Administration of Aqueous Leaf Extracts of *Ficus exasperata*, *Telfeira occidentalis* and *Ficus exasperata/ Telfeira occidentalis* on Haematological Parameters of Chloramphenicol-induced Anaemia in Wistar Rats

Tables 1-3 show the effect of the administration of Aqueous Leaf Extracts of *Ficus exasperata*, *Telfeira occidentalis* and *Ficus exasperata/ Telfeira occidentalis* on haematological parameters of chloramphenicol- induced anaemia in Wistar rats. Aqueous leaves extracts of *Ficus exasperata* and *Telfeira occidentalis* produced significant increase in PCV and Hb with a corresponding decrease in WBC after 7 days of oral administration to anaemic rats

comparable to the reference drug- ferrous gluconate. Percentage recoveries of haematological parameters produced by both plants were also similar. However, co-administration of the extracts synergistically produced an increase in PCV, Hb and a decrease in WBC count after 7 days of oral administration to anaemic rats with effect more than that of the reference drug- ferrous gluconate. The non- anaemic control consisting of untreated rats showed the highest PCV (42.75%) and Hb (12.93g/dl) values. Only the co-administration of the extracts of the two plants nearly restored fully the PCV (41.95%) and Hb (12.89 g/dl) among the treated rats when compared with the untreated non- anaemic rats.

### 4. DISCUSSION

Anaemia is the most prevalent nutritional deficiency disorder in the world. WHO defines anaemia as the condition in which the haemoglobin content of blood is lower than normal as a result of deficiency of one or more essential nutrients [24]. Through the ages man has learnt to take advantage of the many resources placed at his disposal by nature to meet his essential needs in all fields. The diversity of the flora in Africa partly explains the strength of traditional medicine. Many herbs have been used locally for the management of anaemia [25]. Two of such plants are *Ficus exasperata* and *Telfeira occidentalis*. Therefore, this study was undertaken to validate the haematinic potentials of the aqueous leaf extracts of the plants administered separately and a possible synergistic effect when administered concurrently in chloramphenicol-induced anaemic rats.

**Table 1. Effect of the administration of aqueous leaf extracts of *Ficus exasperata*, *Telfeira occidentalis* and *Ficus exasperata/ Telfeira occidentalis* on packed cell volume (%) of chloramphenicol- induced anaemia in wistar rats**

Treatment	Packed cell volume (%)		
	Pre- treatment	Post- treatment	% Recovery
NAC (1ml dist. H <sub>2</sub> O)	-	42.75 ± 3.55	-
AC (1ml dist. H <sub>2</sub> O)	15.16±0.99	15.88±0.74	2.61
FG(900 mg/kg)	17.36±1.05	25.32±2.64	31.35
FEAE (200 mg/kg)	17.48±1.21	22.42±1.42	19.47
TOAE (200 mg/kg)	15.31±1.23	21.25±2.41	21.47
FEAE (100mg/kg) + TOAE (100 mg/kg)	18.23±0.75	41.95±0.37	94.49

Data are presented as mean ± SD, n=5), (-) not treated, NAC= non- anaemic control, NA= anaemic control, FG= ferrous gluconate

**Table 2. Effect of the administration of aqueous leaf extracts of *Ficus exasperata*, *Telfeira occidentalis* and *Ficus exasperata/ Telfeira occidentalis* on haemoglobin (g/dl) of chloramphenicol- induced anaemia in wistar rats**

Treatment	Haemoglobin (g/dl)		
	Pre- treatment	Post- treatment	% Recovery
NAC (1 ml dist. H <sub>2</sub> O)	-	12.93 ± 2.05	-
AC (1 ml dist. H <sub>2</sub> O)	5.51±0.84	5.60±9.71	1.21
FG(900 mg/kg)	7.44±1.59	10.12±2.23	48.82
FEAE (200 mg/kg)	6.25±0.99	9.56±1.11	49.56
TOAE (200 mg/kg)	6.23±0.63	9.48±2.01	48.51
FEAE (100mg/kg) + TOAE (100 mg/kg)	5.51±0.88	12.89±1.29	99.46

Data are presented as mean ± SD, n=5), (-) not treated, NAC= non- anaemic control, NA= anaemic control, FG= ferrous gluconate

**Table 3. Effect of the administration of aqueous leaf extracts of *Ficus exasperata*, *Telfeira occidentalis* and *Ficus exasperata/ Telfeira occidentalis* on white blood count ( $\times 10^3/\text{mm}^3$ ) of chloramphenicol- induced anaemia in wistar rats**

Treatment	White blood count ( $\times 10^3/\text{mm}^3$ )	
	Pre- treatment	Post- treatment
NAC (1ml dist. H <sub>2</sub> O)	-	-
AC (1ml dist. H <sub>2</sub> O)	3.41±0.44	3.51±0.24
FG(900 mg/kg)	6.33±0.51	4.42±0.57
FEAE (200 mg/kg)	5.43±0.26	3.23±0.39
TOAE (200 mg/kg)	5.61±0.81	3.35±0.50
FEAE (100mg/kg) + TOAE (100 mg/kg)	7.70±0.45	3.93±0.62

Data are presented as mean ± SD, n=5), (-) not treated, NAC= non- anaemic control, NA= anaemic control, FG= ferrous gluconate

The results obtained in this study showed that aqueous leaves extracts of *Ficus exasperata* and *Telfeira occidentalis* produced an increase in PVC and Hb level of anaemic rats. Co-administration of the two extracts also produced a synergistic increase in PVC and Hb level. These corroborated previous studies which have shown that such increase in PCV and haemoglobin level of rats after treatment with extracts were obvious indices for recovery from anaemia [26].

The extracts of both plants administered separately and concurrently resulted in fall in the WBC values which might be expected recognizing that chloramphenicol which was used to induce anaemia is noted as a high risk drug which obviously affects the immune system of the experimental rodents leading to a fall in WBC. Prolonged administration of the extract beyond 7 days might restore the WBC. This is in agreement with the report that full recovery of experimental animals from biochemical effects of plant extracts could be achieved by chronic administration of the extracts [27,28].

The interaction between herbs or herbal preparations could either be pharmacodynamic

or pharmacokinetic in nature. A pharmacodynamic interaction occurs when both herbs have affinity for the same receptors or physiological process while pharmacokinetic interaction results when the absorption, distribution, biotransformation or elimination of one herb is modified by another herb resulting in altered pharmacological response. Both pharmacodynamic and pharmacokinetic interactions can lead to additive or synergistic pharmacological activity as observed in this study.

## 5. CONCLUSION

The anti- anaemic effect observed with co-administration of the extracts was significantly more than either of the extract administered alone. It can be concluded that the extracts of *Ficus exasperata* and *Telfeira occidentalis* show synergistic effect when co-administered. This could be useful in the management of anaemia.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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