Effect of a combination of ethanol extract of *Ficus capensis* and *Cnidoscolus aconitifolius* on Liver and Kidney function parameters of phenylhydrazine-induced anemic rats

ABSTRACT

Background: Anemia is a condition in which there is a reduced number of red blood cells or haemoglobin and iron concentration in the body thereby leading to a decreased capacity of the blood to carry oxygen to the body tissues.

Objectives: The study was designed to investigate the effect of a combination of ethanol extract of *Ficus capensis* and *Cnidoscolus aconitifolius* in phenylhydrazine-induced anemic rats.

Methods: The animals were divided into five groups of five rats each. Group A served as normal control, Group B as anemic control, group C as standard drug control, groups D and E were treated with 200mg/kg and 400mg/kg of combined ethanol leaf extract of *F. capensis* and *C. aconitifolius* respectively. Phenylhydrazine was administered intraperitoneally at a dose of 20mg/kg b.w. for two days to induce anemia in rats. The administration of the extract lasted for 14 days after which the animals were sacrificed and blood obtained through cardiac puncture for kidney and liver biomarkers analyses.

Results: Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Total bilirubin, Direct bilirubin and Kidney function parameters assayed in the anemic untreated group showed significant increases ($p<0.05$) compared to the normal rats which may be attributed to toxicity induced by phenylhydrazine. The groups treated with the ethanol extract at a dose of 200 and 400 mg/kg body weight revealed a significant ($p<0.05$) decrease in the liver function parameters compared to the anemic untreated group

Conclusion: This study suggests that combined ethanol leaf extract of *F. capensis* and *C. aconitifolius* is safe and can be effective in the treatment and management of anemia.

Key words: *F. capensis*, *C. aconitifolius*, anemia, liver enzymes, kidney function test.

1. INTRODUCTION

Anemia can be defined as attenuation in the ability of the blood to carry oxygen due to a decrease in the total number of erythrocytes (each having a normal quantity of hemoglobin), a diminished concentration of hemoglobin per erythrocyte, or a combination of both [1]. Anemia is a global public health problem associated with a serious increased risk of morbidity and mortality especially in developing countries in Africa such as Nigeria. It is one of the commonest preventable causes of death in children under 5 years and in pregnant women [2] in poorer malaria-endemic countries, and hence, poses a great threat to global healthcare [3]. Kanfer and Nicol [4] reported that this disease is characterized by the decrease of the hemoglobin rate to less than 13 g/dl in males or 12 g/dl in females.

Albeit there are various drugs used for the treatment of anemia. Many poor people, especially those in the developing countries such as Nigeria cannot have access to them because they are quite expensive, hence, they depend heavily on plants and herbal products for the treatment of diseases and anemia. According to Oladiji [5], it remains a major public health concern in many developing and under developed countries with all age groups at risk, because it causes varying degrees of lowered work capacity, pregnancy complications impairment in cognitive performance, lowered immunity to infections, reduced psychomotor skills, and poor learning capacity. A good number of medicinal plants are traditionally employed to assuage anemia. Some of these plants include *Telfeira occidentallis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavenscens*, *Brillantaisia* was reported to be effective in the treatment of sickle cell anemia [6]. Although the availability of modern medicine may be obtainable in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants are frequently used as raw materials for extraction of active ingredients which are used in the synthesis of different drugs. In the case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants [7].

Ficus capensis commonly known as bush fig, fig of heaven according to [8], is a fast-growing, deciduous or evergreen tree. It usually grows to about 5- 12 metres (16-39 ft) in height, but may attain a height of 35-40 metres (115-131 ft). Almost all the parts of *F. capensis* plant have been found useful. Some parts are used in the treatment of pregnancy-related ailments most especially cases of threatened abortion [9]. The latex is used in the treatment of wounds, eye problems, toothache, general body pain, lung and throat problems, gonorrhoea and as an anti-emetic [10].

Cnidoscolus aconitifolius belong to a group of arbrescent shrubs. It is a drought deciduous evergreen shrub up to 6 m in height with alternate palmate lobed leaves, milky sap and small flowers on dichotomously branched cymes. The leaves are large, 32 cm long and 30 cm wide on chartacious and succulent petioles. The leaves are commonly eaten as vegetable. Rowe [11] reported the use of the shoots and leaves of *C. aconitifolius* as a laxative, diuretic, circulation and lactation stimulants. It has also been recommended for diabetes, obesity, acne, kidney stones and eye problems [12]. Up till date, it has continued to be used as food, medicine and ornamental plant. Potential productivity and above all its substantial nutritional value, the plant has spread all over the world including the tropics due to its ease of cultivation.

Although the synergistic effect of the ethanol extract of *F. capensis* and *C aconitifolius* leaf in the treatment and management of anemia has been studied [13], its effect on the liver and kidney function parameters have not been scientifically evaluated to know its safety in the rising claim of medicinal plants in the clinical management of certain diseases including anemia. Therefore, this study was carried out to ascertain its viability in ameliorating the effects of anemia in phenylhydrazine toxicity when administered as a combination.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *F. capensis* were collected by 10:00am at Ibeagwa Nike, Enugu East Local Government Area, Enugu State. The leaves of *C. aconitifolius* were collected by 12:30pm at Umueze town, Nkanu West Local Government Area, Enugu State.

2.2 Sample Identification

The leaf samples were identified and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The voucher number of *F. capensis* is 164 while that of *C. aconitifolius* is 168. The samples were further deposited at the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka.

2.3 Preparation of the Ethanol Extracts of *F. capensis* and *C. aconitifolius*

The leaves were hand-picked, thoroughly washed and air-dried at room temperature for four weeks. The dried leaves were ground into powder using Corona manual grinding machine. Exactly 300g of the ground leaves powder of *F. capensis* and *C. aconitifolius* were respectively soaked in 1 litres of 80% ethanol for 24 hours for complete extraction. The ethanol extraction was sieved and filtered using Whatman no 1(125mm) filter paper. The filtrates were dried using water bath at 50^0C.

2.4 Test Animals

Male Wistar albino rats were purchased from Chris Animal Farms and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages at the Department of Applied Biochemistry Laboratory, Enugu State University of Science and Technology according to the Institutional Animal Care and Use Committee (IACUC) guidelines on the care and handling of experimental animals. They were allowed to acclimatize for one week before use. The animals were kept on vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state and fed *ad libitum*. At the end of one-week acclimatization period, the animals were weighed, grouped and labeled before the commencement of the research.

2.5 Study Design

The animals were randomized into five (5) groups of five rats each. After the induction of anemia with phenylhydrazine, the animals were treated for two weeks after which blood was collected by cardiac puncture and used for haematological, liver and kidney function analysis. They were grouped as follows: Group A was the normal control, Group B was the Negative Control (Induced but not treated), Group C was Positive Control (Induced and treated with vitamin B_{12}), Group D was Treated with 200mg/kg b.w. of a combination of ethanol extract of *F. capensis* and *C. aconitifolius* and Group E was treated with 400mg/kg b.w. of a combination of ethanol extract of *F. capensis* and *C. aconitifolius*.

2.6 Induction of Anemia

Anemia was induced intraperitoneally in rats using 20mg/kg b.w. of phenylhydrazine daily for two days before the commencement of treatment. The animals were monitored for the symptoms of anemia before the commencement of treatment.

2.7 Liver Function Test

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Bilirubin were determined using Randox diagnostic kits. The procedures used were according to the manufacturer's instruction.

2.8 Kidney Function Test

Urea and creatinine were analysed using Randox test kits. The procedures were according to the manufacturer's instructions.

2.9 Statistical Analysis of Results

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA) and expressed as Mean±SEM. Statistical analysis of the results obtained were performed by using ANOVA and Post-Hoc Tests to determine where significant differences exist between the mean values of the test and control groups. Values at $p<0.05$ were considered significant.

3. RESULTS

3.1 Result of the liver function test

Induction of anemia significantly ($p<0.05$) increased the level of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), direct bilirubin (D. BIL) and total bilirubin (T. BIL) of all the groups except the normal control group that was not induced (Table 1). There was a significant reduction in the ALP, ALT, AST, D. Bil and T.BIL of the groups treated with the extract combination compared to the anemic untreated group. Treatment for a period of fourteen days with graded doses of ethanol extract of *F. capensis* and *C. aconitifolius* normalized the liver function parameters by reducing it to almost the same level observed in the normal control rats.

Table 1: Effect of ethanol extract of a combination of *F. capensis* and *C. aconitifolius* leaf on the liver function parameters of phenylhydrazine-induced anemic rats.

Group	ALP (U/L)	ALT (U/L)	AST (U/L)	D.BIL (mg/dl)	T.BIL (mg/dl)
Normal Control	132.9 ± 0.867	35.37 ± 0.92	57.65 ± 4.667	0.433 ± 0.0915	1.030 ± 0.0255
Anemic untreated	329.4 ± 12.79	57.12 ± 0.43	128.2 ± 3.027	1.105 ± 0.0359	1.975 ± 0.0566
Positive Control ($VitB_{12}$)	133.9 ± 2.83*	37.23 ± 2.43*	57.29 ± 1.395*	0.680 ± 0.0564*	1.280 ± 0.154 *
200mg/kg Ethanol Extract	151.0 ± 22.79*	33.35 ± 1.31*	76.79 ± 4.371*	0.575 ± 0.0479*	1.045 ± 0.0973*
400mg/kg Ethanol Extract	153.2 ± 17.86*	37.79 ± 3.26*	75.69 ± 9.877*	0.664 ± 0.113*	1.005 ± 0.0226*

Result expressed as mean ± SEM; **mean values differ significantly at $p<0.05$.

3.2 Result of the Kidney function test

The result of the kidney function test revealed that the induction of anemia with phenylhydrazine increased the level of creatinine and urea in all the groups except the normal control group (figure 2). Treatment with the ethanol extract of a combination of *F. capensis* and *C. aconitifolius* normalized the creatinine and urea level by reducing their level in all the extract treated groups.

Table 2: Effect of ethanol extract of a combination of *F. capensis* and *C. aconitifolius* leaf on the kidney function parameters of phenylhydrazine-induced anemic rats.

	Creatinine (mg/dl)	Urea (mg/dl)
Normal Control	1.108 ± 0.0517	25.90 ± 1.474
Negative Control	1.283 ± 0.0703	29.40 ± 0.509
Positive Control (Vit B_{12})	1.070 ± 0.0478	25.66 ± 0.647

200mg/kg Ethanol Extract	1.093 ± 0.0621	25.81 ± 0.698
400mg/kg Ethanol Extract	1.150 ± 0.0286	26.21 ± 1.773

Result expressed as mean ± SEM; *mean values differ significantly at $p<0.05$

4. DISCUSSION

PHZ, a non-immunogenic drug that generates changes in the red cell membrane, resulting in oxidative denaturation of hemoglobin (hb), and leading to the formation of an altered Hb called "Heinz bodies" which attenuates the life span of the erythrocytes [14]. This is often distinguished by a significant increase in the incidence of micronucleated polychromated and hypochromic erythrocytes resulting in elevated mean cell volume and decreased mean cell Hb concentration values [15]. According to Waghmare [16], PHZ-induced anemia is a model for the study of hematinic effects.

The presence of iron in the leaves of *F. capensis* and *C. aconitifolius* has earlier been reported. Iron is vital for the synthesis of red blood cells essential for formation of hemoglobin, the oxygen carrying pigment in red blood cells. It is used in the treatment of anemia, tuberculosis and disorder of growth. Also, Iron is an energizer but causes fatigue in high concentrations, although high concentration is hardly taken from natural source. Ndem [17] reported that the availability of iron in diet may modulate the regenerative response. It has earlier been reported that the ethanol extract of a combination of *F. capensis* and *C. aconitifolius* has antianemic property and is capable of replenishing blood in phenylhydrazine-induced anemic rats.

In the results (Table 1) of the liver biomarkers (AST, ALT, ALP, total and direct bilirubin) of the extract treated group (group D and E), there was a significant decrease ($p<0.05$) when compared to that of the anemic group, which was an indication of the damaging effect of the phenylhydrazine to the liver. The damaging effect caused by the induction of phenylhydrazine was restored to near normal in the groups treated with varying doses of a combination of *F. capensis* and *C. aconitifolius*.

The kidney function parameters, creatinine and urea analysed did not reveal any significant difference in the values gotten from the normal group compared to the untreated anemic and treated anemic groups (Table 2). Although there was an increase in the urea level of the anemic untreated group compared to the anemic treated group, the observed difference is not statistically significant.

5. CONCLUSION

It is important to infer that the continuous administration of a combination of ethanol extract of *F. capensis* and *C. aconitifolius*at the ratio of 1:1 for a period of two weeks in phenylhydrazine - induced anemic rats ameliorated the toxic effect of phenylhydrazine by normalizing the liver and kidney function parameters. Further study is needed to fractionate the plant extracts and determine the fraction where the major activity relies and at the same time study the effect of the fractions on the biochemical parameters of the anemic rats.

ETHICAL APPROVAL

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Care and Use Committee

(IACUC) of Nnamdi Azikiwe University, Awka, Nigeria. Efforts were made to minimize all forms of suffering until time of sacrifice of the animals.

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