Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2019; 7(6): 161-166
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Received: 12-09-2019
Accepted: 16-10-2019

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Lipid profile and its complications in phenylhydrazine-induced anemic rats

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Abstract
Objective: The study evaluated the effect of a combination of ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius* on lipid profile of phenylhydrazine-induced anemic rats.
Materials and Methods: Acute toxicity (LD_{50}) was done using Lorke's method. Haematological parameters were analysed using automated haematology analyzer. Lipid profile and random glucose levels were carried out using standard laboratory methods. Twenty-five (25) rats were randomly divided into five groups of five rats each. Group A served as a normal control, Group B as anemic control, Group C as standard drug control, Groups D and E served as test groups treated with 200mg/kg and 400mg/kg body weight of combined ethanol leaf extract of *F. capensi s* and *C. aconitifolius* respectively. Administration of the extract lasted for 14 days after which the animals were sacrificed and blood samples were collected by closed cardiac puncture and used for haematological and lipid profile analysis.
Results: The result of the acute toxicity study showed that the LD_{50} was above 5000mg/kg b.w. thus, the combination of the two extracts was not toxic. Our findings showed significant increase ($p< 0.05$) in haemoglobin concentration, packed cell volume and platelet count of the extract treated groups compared to the anemic untreated group. White blood cells significantly decreased in the extract treated groups compared to the anemic untreated group. The graded doses of the extract combination significantly ($p< 0.05$) increased high density lipoprotein (HDL) while low density lipoprotein (LDL), total cholesterol (TCHOL), triglyceride (TRIG) and very low-density lipoprotein (VLDL) were significantly ($p< 0.05$) reduced compared to anemic untreated group.
Conclusion: The findings from this study suggest that combined ethanol leaf extract of *F. capensis* and *C. aconitifolius* was safe and may be useful in the management of anemia and lipid profile complications resulting from anemia. This may be due to the anti-anemic potentials of the extract and its ability to normalize the lipid profile complications caused by anemia.

Keywords: *F. capensis, C. aconitifolius*, phenylhydrazine, anemia, lipid profile

Introduction
Anemia is a medical condition in which the normal quantity of circulating hemoglobin in the blood is less than 13g/dl for male and less than 12 g/dl for female adults [1]. It is a blood disorder that is defined as either red blood cells (RBC) count below normal, red blood cells which are smaller in size than normal or a level of hemoglobin below normal. The various forms of anemia include iron deficiency anemia; hemolytic anemia; vitamin B_{12} deficiency anemia; folic acid deficiency anemia; anemia caused by inherited abnormalities of RBCs such as sickle cell anemia and thalassemia; and anemia caused by chronic ongoing disease [2]. Anemia is characterized by reduction in circulating red blood cells, haemoglobin and haematocrit unit of peripheral blood [3].
WHO [4] globally estimated that 293.1 million children under five years, approximately 43%, are anemic worldwide and 28.5% of these children are found in sub-sahara Africa. Thus, anemia is a global public health problem affecting both developed and developing countries like Nigeria and it is more prevalent in children under five years and pregnant women. The incidence of anemia is higher in the third world than in the developed countries due to poor nutrition, high prevalence of blood parasites, example include plasmodium, trypanosomes and helminthes infestation [5]. Its consequences are inimical as it affects the cognitive performance,

behavior, physical growth and delayed psychomotor development in children with significant impact on the health of the fetus as well as that of the mother [6]. Anemia is implicated in twenty percent of maternal death in Africa. It exposes fetuses to risk of preterm deliveries, low birth weights, morbidity and prenatal mortality due to the impairment of oxygen delivery to placenta and fetus [7].

Anti-anemic synthetic drugs are mostly not affordable, even when one can afford the drugs, sometimes the side effects are not tolerable. Thus, owing to the difficulties experienced in the management and control of anemia due to poverty, ignorance, and lack of accessible healthcare in most parts of Africa, there is need to study and utilize indigenous medicinal plants with anemic properties that will help to manage this condition both in rural and urban areas especially in Nigeria where there is high prevalence of anemia among children and pregnant women due to insurgency and human displacement.

Lipid is a family of organic compounds that are mostly insoluble in water. Composed of fats and oils and are molecules that yield high energy and have chemical compositions mainly of carbon, hydrogen, and oxygen. They are present in biologic systems mainly as energy stores within cells or as components of cell membranes. The non-polar lipids occur mainly as esters of fatty acids that are virtually insoluble in water and enter metabolic pathways only after hydrolysis. The triacylglycerols (also called triglycerides or fats) are composed of three fatty acids esterified to glycerol [8]. In living organisms especially humans, lipids functions include to store energy; regulate homeostasis and biosignaling; insulate and protect vital organs and tissues; aid digestion and increase bioavailability of essential nutrients. Inside the intestinal cells, the monoglycerides and fatty acids reassemble themselves into triacylglycerols. Triacylglycerols, cholesterol, and phospholipids form lipoproteins (proteins that contains a lipid which serves to transport fat through blood and lymph) when joined with a protein carrier [9]. Lipoproteins have an inner core that is primarily made up of triacylglycerols and cholesterol esters which is cholesterol linked to a fatty acid.

Cnidoscolus aconitifolius (Chaya) is a plant of ancient origin, with a long history of human use, propagation and domestication [10]. In South Western Nigeria, it is known as "Iyana ipaja" [11], whereas in South Eastern Nigeria, it is called "Uno-Ogwueteka (Hospital too far)" [12] because of its blood boosting effects. In South Western Nigeria, the leaves and young shoots are often squeezed with water and drank alone or with milk and tomato paste added. The plant is believed to have a blood-boosting effect, and so is commonly taken by pregnant women and young children who are anemic [12]. Studies have shown that *Cnidoscolus aconitifolius* has ameliorative effects on anemia and osmotic fragility induced by protein-energy malnutrition in male wistar rats [11].

Ficus capensis commonly known as fig tree from *Moraceae* family is a medicinal plant found in terrestrial zones mostly along rivers. *F. capensis* is a spreading deciduous or evergreen tree with a thick bole and spreading roots. It produces fruits throughout the year and the leaves are broad and green. In Nigeria, *F. capensis* has been used in treatment for dysentery and in wound dressing [13]. The leaves and stems' bark of the plant have inhibitory effects against *Esherichia coli* and *Shigella species*. It is also used in herbal medicines to treat threatened abortion, leprosy, epilepsy, rickets, infertility, gonorrhoea, oedema, respirotory disorders and emollient [14]. It is also used in circumcision, leprosy and epilepsy treatment. Some parts are used to treat pregnancy-related ailments most especially cases of threatened abortion [15].

Materials and Methods

Collection and identification of plant samples

The leaves of *Ficus capensis* were collected at Ibeagwa Nike, Enugu East Local Government Area, Enugu State. The leaves of *Cnidoscolus aconitifolius* were collected by at Umueze town, Nkanu West Local Government Area, Enugu State. The samples were validated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. The voucher number of *F. capensis* and *C. aconitifolius* as deposited at the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka. are 164 and 168 respectively.

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. Five hundred (500g) of the ground leaves powder of *F. capensis* and *C. aconitifolius* were respectively soaked in 2 litres of 80% ethanol for 24 hrs for complete extraction. The ethanol extraction was sieved and filtered using Whatman no 1(125mm) filter paper. The filtrate was dried using water bath at 50 ^{0}C. The two extracts were reconstituted with distilled water in the ratio of 1:1 and administered to the experimental subjects.

Chemicals

All the chemicals used in this study were of analytical grade.

Experimental Animals

Male Wistar albino rats weighing between 65 and 80g were purchased from Chris Animal Farms and Research Laboratory, Awka, Anambra State and used for the experiment. They were maintained and housed in cages in the Department of Applied Biochemistry Laboratory, Nnamdi Azikiwe University, Awka. They were allowed to acclimatize with the environment 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 labelled.

Acute toxicity (LD_{50}) testing

Thirteen (13) male wistar albino rats were used for the acute-toxicity testing according to Lorke's method [16]. This involved two phases. The first phase was achieved by giving widely differing low doses to the animals, 10, 100 and 1000 mg/kg b.w. after which the second phase involved administering high doses of 1600, 2900 and 5000 mg/kg b.w. and monitoring for 24 hours for changes in behaviour and mortality.

Animal Grouping, Induction of Anemia and extract administration

Twenty-five (25) male rats of Wistar strains were randomly grouped into five (Groups A-F) groups of five (5) rats each. Group A was left uninduced and served as normal control. Group B to F were induced with anemia intraperitoneally using 25mg/kg body weight of phenylhydrazine. The induction was done for three consecutive days before the commencement of treatment. Group B was left untreated and

served as anemic control. Group C was treated with 5mg/kg body weight of vitamin B_{12}. Groups D and F were continuously treated for 14 days with 200 and 400mg/kg body weight of a combination of ethanol extract of *F. capensis* and *C. aconitifolius* leaves respectively. Blood was collected *orbito rectally* at weekly intervals to check whether the extract administration has effect in the random blood glucose levels of the rats. Blood was collected by closed cardiac puncture under pentobarbital anesthesia at the end of 14 days treatment and used for haematological and lipid profile analysis.

Determination of Weight

The weights of the experimental subjects were checked using electronic weighing balance (Alpha-SRS 130). The weight of the rats was monitored before, during and after the experiment to know whether the combination of the extracts had effect in the body weight of the experimental rats.

Determination of Random blood Glucose Levels

The blood glucose levels of the rats were checked before the induction of anemia and in the course of the treatment using One Touch Glucometer (Life Scan, USA) and Test Strips by. Blood glucose levels were determined by collecting blood *orbito rectally*.

Haematological analysis

Haematological parameters that were analysed include Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Platelets, Neutrophils and lymphocytes. They were determined using automated haematology analyzer (Mindray-BC-28000).

Lipid profile

The lipid profile such as High-Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Total Cholesterol (TCHOL), Triglycerides (TRIG) and Very Low-Density Lipoprotein (VLDL) were determined using Randox test kits [17, 18]. Low density Lipoprotein-Cholesterol (LDL-C) was calculated using a standard formula from [19]. The procedure used was according to the manufacturer's instructions.

Statistical analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA and POST-HOC Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

Results

Acute Toxicity (LD_{50}) Test

The result of the acute toxicity study revealed that the ethanol extract of a combination of *F. capensis* and *C. aconitifolius* leaves was not toxic (Table 1 and 2). The administration of low doses (10, 100 and 1000mg/kg body weight) of the extract combination did not show any visible signs of toxicity in the experimental animals within 24 hours of administration (Table 1). The doses were increased (1600, 2900 and 5000 mg/kg body weight) to check the effect of the extract combination in the experimental subjects (Table 2). However, when these high doses were administered, the rat administered 2900mg/kg body weight was slightly weak while the rat administered 5000mg/kg body weight was weak although no death was recorded within 24 hours of administration.

Table 1: The 24-hour acute toxicity (LD_{50}) of low doses of the combined ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius* in the rats.

Extract Dose (mg/kg body weight)	Mortality
10	0/3
100	0/3
1000	0/3

Number of deaths per group = 0, Number of rats per group = 3

Table 2: The 24-hours acute toxicity (LD_{50}) test of high doses of the combined ethanol leaf extract of *Ficus capensis* and *Cnidosculus aconitifolius* in the rats

Extract Dose (mg/kg body weight)	Mortality
1600	0/1
2900	0/1
5000	0/1

Number of deaths per group = 0, Number of rats per group = 1

Weight

The weight of the rats was checked at the beginning and during the course of the experiment. The weights of the rats increased normally throughout the period of the experiment (table 3). There was a significant increase ($p<0.05$) in the weight of all the groups in the second week of the experiment compared to the weight in the first week and at the commencement of the experiment. However, extract administration did not cause any noticeable increase in weight when compared to other groups that were not treated with the extract.

Table 3: Weight of anemic rats treated with the combined ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius*

Groups	Initial Weight (g) Week 0	Weight (g) Week 1	Weight (g) Week 2
Normal Control	75.02 ± 1.089	84.40 ± 2.873*	130.8 ± 5.906*
Anemic untreated	77.80 ± 4.790	106.2 ± 6.583*	123.8 ± 8.034*
Positive Control ($VitB_{12}$)	68.00 ± 3.066	98.20 ± 3.826*	116.0 ± 6.221*
200mg/kg ethanol extract	74.82 ± 1.497	106.5 ± 2.754*	122.3 ± 8.004*
400mg/kg ethanol extract	73.40 ± 3.043	107.4 ± 4.226*	126.0 ± 5.301*

Table is expressed as mean ± SEM * $p< 0.05$ significant difference compared to week 0.

Random Blood Glucose Levels

The result of the glucose levels reveals that the continuous administration of the extract for a period of 14 days did not cause any observable difference in the random blood sugar levels of the rats (table 4). All the groups maintained normal blood glucose levels in the course of the experiment.

Table 4: Glucose levels of anemic rats treated with the combined ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius*

Groups	Initial Glucose Levels (mg/dl)	Glucose (mg/dl) Week 1	Glucose (mg/dl) Week 2
Normal Control	107.4 ± 4.479	82.09 ± 2.056	98.40 ± 4.479
Anemic untreated	92.40 ± 2.182	87.53 ± 3.142	109.6 ± 6.038
Positive Control ($VitB_{12}$)	92.20 ± 5.324	95.46 ± 0.053	96.00 ± 3.098
200mg/kg ethanol extract	91.60 ± 5.600	92.76 ± 1.523	89.09 ± 6.156
400mg/kg ethanol extract	89.20 ± 3.513	82.52 ± 1.605	98.84 ± 1.749

Table is expressed as mean ± SEM* $p<0.05$ significant difference compared to anemic untreated

Haematological analysis

The haemoglobin concentration and packed cell volume of the groups administered 200 and 400mg/kg body weight of the ethanol extract of a combination of *Ficus capensis* and *Cnidoscolus aconitifolius* leaves significantly increased ($p< 0.05$) compared to the anemic untreated group (Table 5). The haemoglobin levels of the groups treated with the extract combination increased more than that of the normal control and the standard drug. The red blood cells of the groups treated with the extract increased more than that of anemic untreated group, and standard drug group although the increase was not statistically significant (p>0.05). There was a significant decrease ($p< 0.05$) in the white blood cells and neutrophil concentration of the groups treated with the extract compared with the anemic untreated group.

Table 5: Effect of the combined ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius* on haematological parameters of phenylhydrazine-induced anemic rats

GROUPS	HGB (g/dl)	RBC (x $10^{12/L}$)	WBC (x $10^{9/L}$)	PLT (x $10^{9/L}$)	LYMPH (%)	NEUT (%)	PCV (%)
Normal Control	10.43 ± 0.657	5.755± 0.326	8.230 ± 0.354	522.5 ± 13.33	59.75 ± 2.983	31.55 ± 2.273	42.33 ± 0.581
Anemic untreated	8.700 ± 0.607	4.605 ± 0.243	15.31 ± 1.165	322.8 ± 32.36	72.45 ± 4.482	47.33 ± 1.424	39.80 ± 0.480
Positive Control ($VitB_{12}$)	10.45 ± 0.666	5.495 ± 0.526	8.785 ± 1.112*	278.3 ± 6.725	57.90 ± 8.351	36.57 ± 6.077	43.20 ± 2.259
200mg/kg Ethanol Extract	11.80 ± 0.280*	5.505 ± 0.402	9.898 ± 0.975*	372.0 ± 63.47*	55.13 ± 2.595	27.88 ± 3.185*	49.50 ± 1.043*
400mg/kg Ethanol Extract	12.40 ± 0.956*	6.108 ± 0.368	9.040 ± 1.265*	527.8 ± 27.78*	61.65 ± 3.882	28.90 ± 3.276 *	51.55 ± 1.398*

Table is expressed as mean ± SEM* $p< 0.05$ significant difference compared to anemic untreated

Lipid profile analysis

High density lipoprotein cholesterol (HDL-C) concentration significantly increased ($p< 0.05$) in all the groups treated with the extract compared with the anemic untreated group. There was a significant decrease ($p< 0.05$) in the low-density lipoprotein cholesterol (LDL-C), triglycerides (TRIG) and very low-density lipoprotein cholesterol (VLDL-C) concentration of the groups treated with the extract and standard drug compared with the anemic untreated group (Table 6). Total cholesterol (TCHOL) significantly decreased ($p< 0.05$) in the group treated with 400mg/kg body weight of the extract compared with the anemic untreated and normal control groups (Table 6).

Table 6: Effect of the combined ethanol leaf extract of *Ficus capensis* and *Cnidoscolus aconitifolius* on lipid profile of phenylhydrazine-induced anemic rats

Groups	HDL (mg/dl)	LDL-C (mg/dl)	TCHOL-(mg/dl)	TRIG (mg/dl)	VLDL-C (mg/dl)
Normal Control	81.07 ± 2.102	15.56 ± 11.49	79.30 ± 7.350	74.11 ± 6.663	14.82 ± 1.333
Anemic untreated	34.85 ± 3.969	86.59 ± 9.365	87.41 ± 4.357	170.1 ± 28.76	34.03 ± 5.751
Positive Control ($VitB_{12}$)	72.77 ± 3.179*	13.11 ± 3.959*	67.20 ± 7.105	84.43 ± 10.68*	18.69 ± 0.693*
200mg/kg Ethanol Extract	64.87 ± 9.962*	30.27 ± 16.42*	73.98 ± 11.43	105.8 ± 4.354*	21.16 ± 0.871*
400mg/kg Ethanol Extract	65.51 ± 8.026*	9.640 ± 10.86*	56.04 ± 6.590*	95.48 ± 6.953*	19.10 ± 1.390*

Table is expressed as mean ± SEM* $p<0.05$ significant difference compared to anemic untreated

Discussion

In this study, phenylhydrazine was used to induce hemolytic anemia in wistar albino rats. Phenylhydrazine is toxic for the body and impairs several tissues, it can cause lysis of red blood cells and production of free radicals leading to oxidative stress, lipid peroxidation and oxidative degradation of spectrum cell membrane [20]. Anemia upstaged the lipid profile of experimental subjects. This can be restored to normal by treatment with medicinal plants. Our study showed that Phenylhydrazine does not cause any significant difference in the weight of the experimental subjects.

The result of the acute toxicity (LD_{50}) study revealed that the combination of the ethanol extract of *F. capensis* and *C. aconitifolius* was not toxic as the LD_{50} value is above 5000mg/kg body weight [16]. The result also showed that the induction of anemia and treatment with the extract did not cause any significant difference in the weight of the rats (Table 3). However, the weight increased normally in the course of the experiment. The results obtained from the glucose levels showed that the extract did not cause any noticeable difference in the random blood glucose levels of the rats (Table 4). This suggests that induction of anemia and its treatment does not impair glucose homeostasis.

Our findings revealed that rats in group A (Table 5) which served as the normal control and which were not induced with anemia or treated had haematological parameters within normal range throughout the duration of the experiment. The haematological parameters, haemoglobin. Red blood cells, packed cell volume, in the anemic group showed a significant decrease ($p< 0.05$) when compared to the normal control group. This could be as a result of the toxicity induced by phenylhydrazine, by peroxidation of red blood cells membrane lipids and this effect may be a result of the auto-oxidation of the drug and the interaction of oxygen radicals with membrane lipids as reported also by Jain and Subrahmanyam [21]. However, in the extract treated group, there was a significant ($p< 0.05$) increase in the hematological parameters when compared to the anemic group. This may be due to the presence of phytochemicals in the plant extract which are well known hemopoietic factors that have direct

influence on the production of blood in the bone marrow and also presence of appreciable amount of iron content [15].

Also, our results revealed that there was an increase in the lymphocytes, neutrophils and white blood cells in the anemic group when compared to the extract treated group and normal control group, which are indicators of leukocytosis, thus describing the anemia as leukocytic. Our investigation on white blood cells showed that there was a significant increase ($p< 0.05$) in the white blood cell levels of the anemic groups when compared to the normal control group and the extract treated groups. This may imply that the extract possesses some potentials that are capable of boosting the immune system in rats which increases the ability of the rats treated with the extract to resist infection. The presence of elevated numbers of neutrophils in the circulation is associated with poor outcome in disease conditions [22]. The results also showed a decrease in the platelet count of the anemic group when compared to the normal control group. However, there was a significant increase ($p< 0.05$) in platelet count in extract treated group when compared to the anemic group, indicating that the extract has a stimulating effect on platelet production.

Our lipid profile findings revealed that rats that were not anemic (normal control group) showed normal range of lipid profile parameters when compared to untreated anemic group. There were significant ($p< 0.05$) increase in HDL level of the combined-extracts treated rats when compared with the values of untreated anemic rats. Also, there were significant ($p< 0.05$) reduction in the values of LDL, TCHOL, TRIG and VLDL of the combined-extracts treated rats when compared with the values of untreated anemic rats. This could be as result of the anti-anemic effect of the combined extracts. The ability of the combined-extract to increase HDL level and decrease LDL level indicates that the combined leaf extracts of *F. capensis* and *C. aconitifolius* may serve as potential agent to regulate the HDL/LDL level in living organism. The significant increase ($p< 0.05$) in LDL, TCHOL, TRIG and VLDL of the anemic untreated rats is in line with the findings of Drechsler *et al.* [23] who reported that Hyperlipidemia and hypercholesterolemia induce neutrophilia, which is positively associated with atherosclerotic plaque burden. This can be the reason while neutrophil level in the anemic untreated rats was also high.

It is worthy to note that LDLs carry cholesterol into cells for normal physiological functions and usage, but at times, LDLs can also deposit cholesterol into the walls of blood vessels, which can lead to harmful cardiovascular disease; conversely, HDLs scavenge the excess cholesterol from the cells, tissues, and blood vessels and deliver same back to the liver, where they are either reused or excreted to avoid causing harm to the organism. The results of the lipid profile is in line with the reports of Igbodaro and Omole [24] who stated that plant extract may likely contain anticholesterolemic substance necessary for the management of lipid peroxidation and its complications.

Conclusion

The study revealed that the combined ethanol extract of *F. capensis* and *C. aconitifolius* has considerable anti-anemic activity and potential to regulate the HDL/LDL level in living organism indicating the use of these plants for the treatment of anemia and may be a good agent to reduce or regulate lipid profile complications in higher animals. Further studies are required to evaluate and characterize the bioactive compounds responsible for the anti-anemic and lipid profile regulatory potentials of the plants.

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