

Effect of *Gossypium hirsutum* L. Ethanol Leaf Extract on Phenylhydrazine-induced Anaemic Rats

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Abstract

The study evaluated the effect of ethanol leaf extract of *Gossypium hirsutum* L. on some haematological indices in Phenylhydrazine induced anaemia in rats. In this study, forty two (42) rats were grouped into seven groups. Anaemia was induced using Phenylhydrazine, a substance which induce anaemia as a result of peroxidation of erythrocytes membrane lipid. The result showed that the ethanol leaf extract of *G. hirsutum* administered orally (100-400 mg/kg body weight) significantly ($p < 0.05$) exhibited hematinic activity in a dose dependent manner on haematological indices compared to experimental control and normal rats. Studies on the serum biochemical markers of liver damage showed significant ($p < 0.05$) protective effect of the extract on treatment groups compared to experimental control and normal rats. The oral LD₅₀ of the ethanol leaf extract was estimated to be greater than 5000 mg/kg body weight indicating it's safe. The results showed that *G. hirsutum* leaf has hematinic properties thus justifying its use in the management of anaemia especially among pregnant women in the traditional medical practice.

Keywords: Phenylhydrazine, Anaemic rats, Ethanol Leaf Extracts, *Gossypium hirsutum*, Anaemia

INTRODUCTION

Anaemia is a medical condition in which the normal quantity of circulating hemoglobin in the blood is less than 13 g/dl for male and less than 12 g/dl for female adults (Okochi *et al.*, 2003). It is characterized by reduction in circulating red blood cells, haemoglobin and Haematocrit unit of peripheral blood (Ubong, 2004; Trease and Evans, 2009). Anaemia is a global public health problem affecting both developed and developing countries, being more prevalent in children under five years and pregnant women. The global estimate indicates that 293.1 million children under five years, approximately 43%, are anemic worldwide and 28.5% of these children are found in sub Saharan Africa. Normal haemoglobin distributions vary with age, sex, and physiological status, (WHO, 2001b)

Anaemia is an important cause of morbidity and mortality in many parts of the world. The burden is higher in Sub Saharan Africa where it has been associated with an increased risk of morbidity and mortality. The consequences of anaemia in children are inimical as it affects their cognitive performance, behavior, physical growth and delayed psychomotor development (Brabin *et al.*, 2001). In pregnancy; anaemia has a significant impact on the health of the fetus as well as that of the mother. Twenty percent of maternal deaths in Africa have been attributed to anaemia. Fetuses are at risk of preterm deliveries, low birth weights, morbidity and prenatal mortality due to the impairment of oxygen delivery to placenta and fetus. The management and control of anaemia in pregnancy seems to be difficult due to poverty, ignorance, and lack of assessable healthcare in most rural area of the country.

Anaemia is a medical condition that contributes to high fatality rate in pregnant women and children under five in both urban and rural areas. The need to study medicinal plants with anaemic properties will help manage this condition both in rural and urban areas especially in the north eastern Nigeria a time where there is high prevalence of anaemia among children and pregnant women due to insurgency. Synthetic drugs are mostly not affordable, making the management of the disease condition very difficult as a result of poverty and displacement. Even when one can afford the drugs sometimes the side effects are not tolerable.

Kannan *et al.* (2009) reported that *G. hirsutum* fruits contained complex antibiotic compounds which cured various diseases like cancer, cardiovascular and digestive diseases. Also, Gossypol obtained from *G. hirsutum* seeds has been reported by Sotelo *et al.* (2005) to have reversible anti-fertility effects in men. Fasola *et al.* (2011) confirmed that water extracts of *G. hirsutum* leaves have shown promising but differential in-vitro antiviral activities and recommended that application of the extracts could help in the treatment of yellow fever infections.

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future (David *et al.*, 2006).

MATERIALS AND METHOD

Materials

Plant Materials

Gossypium hirsutum leaf was collected from Ngurore, Yola south local Government area of Adamawa State in the months of May 2015 and was authenticated by a Botanist at the Department of Plant Science, Modibbo Adama University of Technology, Yola, Adamawa State.

Animals for experiment

A total of fifty two (52) Male Wister rats weighing between 90-120g were purchased from National Veterinary Research Institute, Vom, Nigeria. The animals were housed in a plastic cage and allowed to acclimatise for 7 days and fed with standard diet (Finisher vital feeds Jos) and water *ad libitum*, forty two were divided into seven groups of six each and ten used for LD₅₀.

Methods

Plant extraction

Fresh leaf of *Gossypium hirsutum* was allowed drying at room temperature under shed. Dried plant was made into powder using mortar and pestle, 500 g of the dried sample was cold extracted using 1500 ml of ethanol over 48 hours period. The extract was then filtered using a filter paper (Whatmann No. 1) and concentrated using water bath at 50°C (Bello *et al.*, 2011).

RESULTS

Table 1 shows the effect of the ethanol leaf extract on liver markers. The results show significant decrease in a dose dependent manner with 400 mg/kg body wt. having the highest effect compared to the experimental control. The result also shows a significant decrease in the levels of the liver enzymes compared to the experimental control.

Table 2 shows the effect of the ethanol leaf extract on haematological parameters. Administration of the ethanol leaf extract evoked a significant (P<0.05) increase in the parameters compared to the experimental groups. Significant increase was observed in the values of RBC, Hb, PCV, Neutrophils and platelets, while significant decreases were observed in WBC, Lymphocytes, MCV, MCH, and MCHC values. Treatment with 400 mg/kg body wt. was found to be more effective compared to other extract doses. Significant (p<0.05) decrease in Hb concentration (9.56 ± 0.28 g/dl), RBC (7.28±0.11x10⁶ (μl)), WBC (3.70±0.30 x10³ (μL)) and Haematocrit (24.70 ± 0.35%), was observed in the experimental control compared to treatment group.

Table 1: Effect of Ethanol Leaf Extract of *G. hirsutum* on Serum Biochemical Markers of Liver Damage In Phenylhydrazine induced Hemolytic Anaemia in Rats

Treatment	AST(U/L)	ALT(U/L)	TP (g/l)	CB(mg/dl)	TB(mg/dl)
Normal control	32.00 ± 0.01 ^c	14.97 ± 0.09 ^c	56.03 ± 0.12	0.23 ± 0.00 ^c	4.15 ± 0.07 ^c
Expt. control	57.31 ± 0.35	21.00 ± 0.02	18.95 ± 0.05	1.94 ± 0.02	9.45 ± 0.01
Standard control	51.43 ± 0.47 ^a	20.53 ± 0.27	41.07 ± 0.15	1.45 ± 0.04	7.85 ± 0.03 ^a
AN +100 mg/kg b.w E.E.	47.09 ± 0.27 ^{ab}	19.98 ± 0.08	39.93 ± 0.23	1.31 ± 0.02 ^a	9.06 ± 0.02
AN + 200 mg/kg b.w E.E.	39.96 ± 0.07 ^{ab}	18.90 ± 0.06 ^{ab}	38.60 ± 0.28 ^b	1.17 ± 0.01 ^{ab}	8.32 ± 0.09
AN +300mg/kg b.w E.E.	31.08 ± 0.13 ^{ab}	16.92 ± 0.23 ^{ab}	35.06 ± 0.07 ^b	1.02 ± 0.01 ^{ab}	6.52 ± 0.03 ^a
AN + 400mg/kg b.w E.E.	27.97 ± 0.08 ^{ab}	15.93 ± 0.04 ^{ab}	31.67 ± 0.33 ^b	0.91 ± 0.01 ^{ab}	5.52 ± 0.15 ^{ab}

KEYS: E.E = Ethanolic Extract, AN = Anaemia, All values are mean of six determinations ± SEM, ^a significantly (p< 0.05) lower compared to experimental control; ^b significantly (p< 0.05) lower compared to standard control; ^c significantly (p< 0.05) lower compared to experimental control

Table 2: Effect of Ethanolic Leaf Extracts of *G. hirsutum* on Haematological Indices in Hemolytic Anaemic Rats

Treatment	PCV (%)	Hb (g/dL)	WBC (x10 ³ /μL)	RBC (x10 ⁶ /μL)	Neutrophils (%)	Lymphocyte (%)	Platelets (mm ³).
Normal control	38.36 ± 0.59 ^a	11.43 ± 0.14	7.73 ± 0.08 ^c	7.28±0.11 ^a	52.66±0.88	38.66±0.88 ^d	885.33±1.76
Expt. Control	24.70 ± 0.35 ^d	9.56 ± 0.28 ^d	32.06±0.60	3.70±0.30 ^d	12.33±0.88	88.00±0.58 ^b	448.66±3.38 ^d
Standard control	50.86 ± 0.47 ^a	12.40 ± 0.05 ^a	26.30±0.17 ^c	6.08±0.07 ^a	41.66±0.87 ^a	61.00±0.57 ^c	511.00±0.57 ^a
AN+100 mg/kg b.w E.E.	51.96 ± 0.95 ^a	12.83 ± 0.06 ^a	25.16±0.27 ^c	6.48±0.02 ^a	34.67±0.88 ^{ad}	76.00±0.57 ^{bc}	497.00±2.64
AN+200 mg/kg b.w E.E.	60.56 ± 0.74 ^{ab}	14.10 ± 0.05 ^a	24.20±0.55 ^c	6.83±0.08 ^a	36.30±0.66 ^{ad}	74.00±0.57 ^{bc}	522.33±1.20 ^{ab}
AN+300 mg/kg b.w E.E.	64.10 ± 0.52 ^{ab}	14.03 ± 0.15 ^{ab}	20.36±0.44 ^d	6.91±0.10 ^a	38.00±0.57 ^a	70.00±0.57 ^{bc}	662.33±1.20 ^{ab}
AN+400 mg/kg b.w E.E.	64.30 ± 0.45 ^{ab}	16.43 ± 0.14 ^{ab}	19.25±0.14 ^{cd}	7.00±0.02 ^a	45.33±1.20 ^a	63.00±0.57 ^{bc}	766.67±0.88 ^{ab}

KEY: E.E = Ethanolic Extract, AN= Anemia, All values are mean of six determinations ± SEM, ^a significantly (p< 0.05) higher compared to experimental control; ^b significantly (p< 0.05) higher compared to standard control; ^c significantly (p< 0.05) lower compared to experimental control; ^d significantly (p< 0.05) lower compared to standard control.

Table 3: Effect of Ethanolic Leaf Extract of *G. hirsutum* on Hematological Indices in Hemolytic Anaemic Rats

Treatment	MCV (fL)	MCH (pg)	MCHC (g/dl)
Normal control	57.63 ± 0.53 ^d	16.60 ± 0.10	25.10 ± 0.00 ^c
Expt. control	94.36 ± 0.27 ^b	19.47 ± 0.14 ^b	29.10 ± 0.15 ^b
Standard control	62.70 ± 0.40 ^c	16.43 ± 0.10 ^c	24.40 ± 0.21 ^c
AN.+100 mg/kg b.w E.E.	90.46 ± 0.20 ^b	18.10 ± 0.51 ^b	23.27 ± 0.17 ^c
AN.+200 mg/kg b.w E.E.	82.76 ± 0.52 ^{bc}	17.56 ± 0.32 ^c	23.00 ± 0.40 ^{cd}
AN.+300 mg/kg b.w E.E.	77.50 ± 0.46 ^{bc}	16.60 ± 0.05 ^c	22.10 ± 0.10 ^{cd}
AN.+400 mg/kg b.w E.E.	61.56 ± 0.36 ^c	15.93 ± 0.08 ^c	21.53 ± 0.35 ^{cd}

KEYS: E.E = Ethanolic Extract, AN= Anemia, All values are mean of six determinations ± SEM, ^a significantly (p< 0.05) higher compared to experimental control; ^b significantly (p< 0.05) higher compared to standard control; ^c significantly (p< 0.05) lower compared to experimental control; ^d significantly (p< 0.05) lower compared to standard control.

DISCUSSION

Anaemia is a disease condition characterized by reduction of normal quantity of circulating hemoglobin in the blood less than 13 g/dl for female and less than 12 g/dl for male adults (Okochi *et al.*, 2003). Epidemiological studies indicated that WHO estimates for the number of anaemic people globally for the year 2004 was 2 billion, representing 30% of the world's population (Adusi-Poku *et al.*, 2008). Also over 50% of pregnant women and over 40% of infants worldwide are anaemic with a prevailing significant morbidity and mortality particularly in the developing country (Holden and Acomb, 2007). Hence anaemia is one of the leading health disorders posing a great threat to global healthcare. The study aimed at evaluating the effect of ethanol leaf extract of *G. hirsutum* leaf extract on Phenylhydrazine induced anaemia in rats, phytochemical constitutes mineral analysis and vitamin concentration.

These hematinic agents may have contributed in the faster reversal of the PHZ hemolytic anaemia in the treated rats within the first two weeks of treatment which exhibited progressive recovery of the blood parameters.

Anaemia has many causes, including those of nutritional origin, of which iron deficiency dominates, infectious origin (e.g., due to hookworm, malaria, HIV and other infectious diseases), and those due to inflammation of chronic disease. While iron deficiency is the leading cause of nutritional anemia, vitamin A joins several hematinic nutrients including vitamins C, E, and B₁₂ and folic acid that, when deficient, can adversely affect iron-dependent erythropoiesis and contribute to anemia (Fishman *et al.*, 2000). Vitamin A deficiency, for example, may compromise iron absorption, storage, transport and delivery to bone marrow through several paths (Fishman *et al.*, 2000; Semba *et al.*, 2002), vitamin A deficiency can also reduce erythrocytosis by lowering erythropoietin production (Fisher 2003; Zimmermann *et al.*, 2006).

Administration of ethanol extract of *G. hirsutum* revealed significant decrease in the activities of serum liver enzyme markers AST and ALT as compared to the experimental control. It has been reported that Phenylhydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species, however, antioxidants such as Vitamin A, E, C, flavonoids and phenols are known to reduce the levels of free radicals thereby protecting the cells or repair damage done to red cells by free radicals or highly reactive oxygen species (Clemens *et al.*, 1984). Treatment with the extract might have exhibit some antioxidant activity due to the presence of Phytochemical and detoxifying ability of the rats' liver challenging the Phenylhydrazine damaging effect as when compared with the experimental control group. Decrease in Alanine Transaminase (ALT) and Aspartate Transaminase (AST) mean values suggests that the plant has hepatoprotective properties. The elevated level of the conjugated bilirubin (CB) and total bilirubin (TB) signifies liver injury, but there was a significant decrease in the levels of CB and TB indicating recovery of the liver, as also seen in the liver enzymes markers.

The results of haematological tests indicated that experimental control rats induced with Phenylhydrazine had a significant decrease in Hb concentration, RBC, WBC and Haematocrit. While WBC, Lymphocytes were elevated, indicating anaemia. The administration of *G. hirsutum* leaf extract evoked a significant (P<0.05) increase in the haematological parameters in the experimental groups in a dose dependent manner, Significant increase was indicated in the values of RBC, Hb, PCV, Neutrophils and platelets, and significantly decreased in WBC, Lymphocytes, MCV, MCH, and MCHC.

White blood cells, lymphocytes and neutrophils are indices of immunology of the body against infection. In an event of exposure of the body to any xenobiotic substance it may lead to immunological compromise. The significant increase in WBC, lymphocytes and decrease in platelets and neutrophils is as result of presence of the Phenylhydrazine used in the induction of anaemia. There was a significant increase in the RBCC, Hb, and PCV, after the ethanolic leaf extract was administered. Treatment with 400 mg/kg/ body weight was more effective

than other dose of the experimental groups. The result indicates that the ethanol leaf extract of *G. hirsutum* have anti anaemic potentials and this might be due to the Phytochemical, Vitamin and other mineral constituents necessary for erythropoiesis such as iron, in the plant extract.

CONCLUSION

Studies on the effect of ethanol leaf extract of *G. hirsutum* on Phenylhydrazine induced anaemic rats showed that the extract significantly ($p < 0.05$) exhibited hematinic activity in a dose dependent manner when compared to experimental control and normal rats.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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