

Terminalia catappa Extract Enhances Erythropoiesis in Adult *Balb C* Mice

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Abstract

Anemia and low haemoglobin levels are complications frequently associated with Sickle cell disease and β -thalassaemia. Chemotherapy of these haemoglobinopathies involves the use of drugs which increase haemoglobin levels. The use of *Terminalia catappa* traditionally for the treatment of SCD dates back. Herein we evaluated the potential of *Terminalia catappa* to induce erythropoiesis in adult *Balb C* mice. The methanolic extract of *Terminalia catappa* induced production of haemoglobin higher than that of an untreated control after 6 days. The PCV of treated and untreated mice was also assessed and found to be relatively higher in *Terminalia catappa* treated mice comparable to mice administered with folic acid.

Keywords: *Terminalia catappa*, Erythropoiesis, Sickle cell disease, Balb C mice

1. Introduction

Sickle cell anaemia is a genetic disorder which results from a mutation in the 17th nucleotide of the β -globin gene that leads to the substitution of glutamic acid by Valine as the 6th amino acid of the β -chain of haemoglobin (Bindon, 2004). The disease is characterized with symptoms of varying degree, including haemolytic anaemia as a result of polymerization of the red cells in situations of low oxygen tension. The anti-i-chemical properties of haemoglobin-S form the basis for sickle cell related morbidity. Intra-erythrocytic hypoxia causes haemoglobin polymerization with associated change in red cell shape-sickling. The sickle red cells are rigid with an increased tendency to stick to endothelial surface. Erythrocyte adhesion to endothelium is the basis for vaso-occlusion

manifested as bone pain, strokes, chest syndrome and priapism. Since the Haemoglobin-S containing erythrocytes are more prone to haemolysis, hence sickle cell disease patients are frequently anaemic (Aliyu *et al.*, 2007; Bownas, 2002; Bindon, 2004).

Sickle cell anaemia is a lifelong medical disorder of public health significance in most parts of the world (Weatherall 2010; Chowning 2000).

The use of herbs for the treatment of ailments is as old as man. Several herbs have been used to treat sickle cell disease traditionally (Elujoba *et al.*, 2005).

Terminalia catappa (almond) one plant commonly used to treat sickle cell disease in Nigeria, has been shown to inhibit osmotically induced haemolysis. (Mgbemene *et al.*, 1999). The plant which is also commonly called umbrella tree in northern Nigeria because of the shade the tree provides belongs to the family Combretaceae, and is known to originate from subtropical and tropical zones of India and Pacific oceans (Thomson and Evans 2006). Almond plants have various traditional medicinal uses; the leaves, and fruit are used for treatment of pains and headache in Sri Lanka, dressing of rheumatic joints in Indonesia and India; the bark is used as a diuretic and cardiogenic, the leaves are also used for treatment of headache. In Nigeria, leaves macerated in palm oil used for anti-inflam; stems and bark used for sexual dysfunction. Seeds have been used for sexual dysfunction (<http://www.stuartxchange.com/Talisay.html>). It is also used to treat eye problems in Samoa and to stop bleeding during teeth extraction in Mexico (<http://www.fishforums.net/index.php?topic/41676-indian-almond-leaf/>). The ethanol extract of the leaf show potential in the treatment of sickle cell disease (<http://www.fishforums.net/index.php?topic/41676-indian-almond-leaf/>). Studies have shown that leaf extracts of *Terminalia catappa* have potential to prevent metastasis (Chu *et al.*, 2007), have antioxidant, hepatoprotective, antimicrobial, antiparasitic and antiinflammatory properties among others (Kinoshita *et al.*, 2007; Babayi *et al.*, 2004; Fan *et al.*, 2004).

In spite of several studies on the medicinal properties *Terminalia catappa* (Chu *et al.*, 2007; Kinoshita *et al.*, 2007; Babayi *et al.*, 2004; Fan *et al.*, 2004), Its ability to stimulate red blood cells (RBC) production *in vivo* has not been reported even though it is used as a long term efficacious traditional therapy for SCD. The dearth of scientific validation of plant based sickle cell therapies as reported by Okpuzor *et al* (2008) is the thrust of investigating the efficacy of *Terminalia catappa* methanolic extract on erythropoiesis in Adult Balb C mice.

2. Materials and Methods

T. catappa leaves were obtained fresh in the early hours of the morning and identified at the Ahmadu Bello University herbarium. The fresh leaves were air dried at room temperature within the laboratory.

2.1 Plant extraction

The dried leaves of *T. catappa* leaves were pulverized using a mortar and pestle. 10g of the pulverized leaves was then exhaustively extracted using methanol at boiling point. The extract was then allowed to cool to room temperature and then cryo-concentrated.

2.2 Animal experiments

The concentrated extract was reconstituted in PBS pH 7.2 and administered intraperitoneally to Balb C mice at a dosage of 80mg/kg bodyweight. LD₅₀ of plant extract was previously determined (results not shown) in this lab. Here, the lethal dose of the extract was carried out as described by Lorke 1983 to ascertain the number of surviving animals at 50%. The animal grouping consisted four mice per group and the groupings were; a positive control (administered with folic acid 80mg/ml), negative control (only PBS administered) and test group (administered with 80mg/kg *T. catappa* extract). The administration was allowed to run for 7 days with administration being carried out daily. At the end of the 7th day mice were sacrificed and blood collected in EDTA. The haemoglobin, PCV and WBC levels were determined.

2.3 Haemoglobin assay

Haemoglobin was assayed as previously described Khalid *et al.*, (2003) with slight modifications. Briefly; cells were washed twice with phosphate-buffered saline (PBS), resuspended in 0.5 mL distilled water, and lysed under hypotonic conditions for 10 minutes. The cell lysate was centrifuged at 10 000 rpm in a microfuge for 15 minutes at 37 °C. 200µl of the supernatant was used for the assay. The assay mixture was prepared in duplicate by adding the reagents in the following order: 100 µL supernatant, 900µL distilled water, 100µL freshly prepared benzidine-HCl (10 mg/mL), 40µL of 30% H₂O₂. The contents were mixed well and after exactly 90 seconds the absorbance at 604 nm was measured in a Shimadzu UV-Vis spectrophotometer. Hemoglobin concentration was

determined by comparing this absorbance value to a standard curve prepared using pure human hemoglobin solutions.

2.4 UV spectral scan determination

The concentrated extract was reconstituted in methanol and diluted three folds. 3ml of the dilute extract was scanned in a Shimadzu UV-visible spectrophotometer. Wavelength range of scan was between 200-800nm.

2.5 Phytochemical analysis

This was carried out according to the method of Trease and Evans (1989) as previously described by Harborne (1973) and modified by Edeoga (2005).

2.6 Statistical analysis

The means of the different groups were compared using the Student t-test.

3. Results

3.1 Terminalia catappa improves intracellular hemoglobin

Erythropoiesis was studied by monitoring the level of intracellular haemoglobin in both untreated and *Terminalia catappa* treated Balb C mice and compared with that of the control. The level of intracellular haemoglobin was significantly higher in *Terminalia catappa* treated mice ($P < 0.05$) as compared to the untreated control Balb C mice (Figure 1). There was no significant difference ($P < 0.05$) between the intracellular hemoglobin levels of both the *Terminalia catappa* treated and Folic acid treated groups.

3.2 Terminalia catappa induces production of total formed elements of blood

The ability of *Terminalia catappa* to improve the overall formed elements of the blood was also assessed by determining the effect of *Terminalia catappa* extract on the PCV of adult Balb C mice 7 days post treatment of the mice using *T. catappa* extract.

Terminalia catappa induced a significantly higher percentage increase ($P < 0.05$) in PCV (Table 1) of mice 7 days post treatment comparable to folic acid as opposed to the untreated control. The relative increase in PCV in mice treated with *Terminalia catappa* is shown in Figure 2.

3.3 Phytochemical profile of Terminalia catappa

The phytochemical content of *Terminalia catappa* extract revealed the presence of alkaloids and anthraquinones (Table 2).

3.4 UV-visible absorbance spectra of methanolic extract of Terminalia catappa

The spectra was carried out at every 0.5 interval in a 1cm quartz cuvette, covering a scan range of 200-800nm. The spectra revealed the presence of several prominent peaks at 260, 334, 418, 435, 468, 615 and 664nm as shown in Figure 4.

4. Discussion

The management of sickle cell disease involves the use of chemical agents that enhance erythropoiesis to ameliorate the symptoms of anaemia. *Terminalia catappa* has been used for many years in northern Nigeria to manage sickle cell anaemia. herein we investigated the effect of the extract of *Terminalia catappa* on Balb C mice intracellular haemoglobin and PCV level. The plant extract was therapeutic at a dose of 80mg/kg body weight of adult Balb C mice. The plant extract induced intracellular haemoglobin levels significantly above the untreated control and comparable to that of the folic acid, this could be one of the medicinal benefits of the plant which has enabled its use over time locally.

The precise mechanism by which the extract induces erythropoiesis can not be said at this point, but the findings is consistent with previous studies by Mgbemene *et al.*, 1999 which demonstrated that *T. catappa* was able to inhibit osmotically induced haemolysis. Thus it is possible that the extract posses some haematopoietic protective effect.

In order to ascertain the haematopoietic inductive potential of *T. catappa* we assessed its effect on the PCV of Balb C mice before and after treatment of the mice. The extract showed 16.67% induction of the PCV level of the mice close to Folic acid which revealed a 25% increase in PCV of the mice on the other hand the untreated control showed an almost steady PCV level during the 7 day experimental period. This could be important in the management of sickle cell anemia since anaemia as revealed by a much lowered PCV levels in sickle cell anaemia patients is one of the major ways in which the disease is expressed (Buchanan *et al.*, 2004). Although further studies is needed to understand the mechanism behind these induction.

We also monitored the effect of the extract on the weight of the mice to see if the extract enhances feeding in mice this revealed no effect of the extract on the animals in feeding behavior of the extract, as there was a slight decrease in the bodyweight of the mice after the 7 days experimental period as shown in Figure 3.

The phytochemical content revealed the presence of alkaloids and anthraquinones which could be responsible for the observed effects of the plant extract, the UV-Visible absorbance spectra also revealed distinct peaks which could represent characteristic compounds which would require further studies for complete characterization. These plant components could be acting in synergy to elicit their haematopoietic and erythropoietic effects but this would also require further characterization.

In summary, methanolic extract of *Terminalia catappa* revealed an erythropoietic potential in adult Balb C mice. The extract induced intracellular haemoglobin levels higher than an untreated control and comparable to that of a standard erythropoietic drug Folic acid the extract also showed haematopoietic potential being able to increase the PCV of the mice significantly above the control 7 days post treatment of the mice with the extract. The phytochemical and UV-Visible spectra reveal the presence of alkaloids and anthraquinones. More studies is required to ascertain the precise mechanism in which the methanolic extract of *Terminalia catappa* elicits its erythropoietic effect in Balb C mice and also the active principles involved which can enhance plant based drug development.

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Table 1. Percentage Induction of PCV of adult Balb C mice after 7 days treatment with methanolic extract of *Terminalia catappa* Folic acid was used as control drug and compared with an untreated control. PCV was determined using the capillary technique and measured using a haematocrit reader

Folic acid	25% ± 2.5
Untreated control	2.33% ± 2.0
<i>Terminalia catappa</i>	16.67% ± 1.6

Table 2. phytochemical content of methanolic extract of *Terminalia catappa* extract

	Phytochemical	Status
1	Saponins	Absent
2	Alkaloids	Present
3	Flavonoids	Absent
4	Anthraquinones	Present
5	Glycosides	Absent

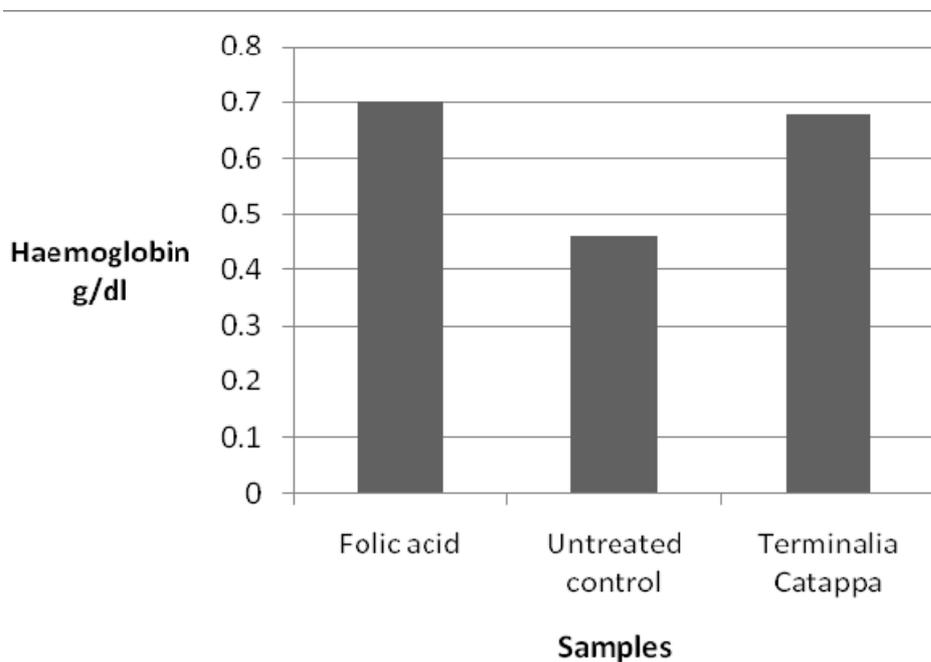


Figure 1. *Terminalia catappa* induced haemoglobin levels higher than the untreated control and comparable to Folic acid. Intracellular haemoglobin levels were measured after 7 days of treatment with extract by the benzidine spectrophotometric assay using human haemoglobin as standard

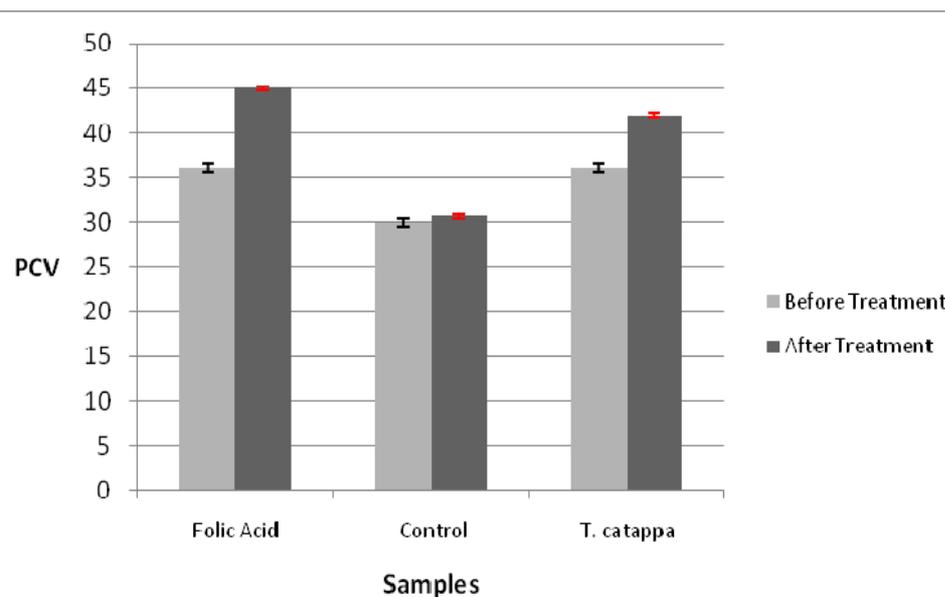


Figure 2. *Terminalia catappa* improves PCV levels in adult *Balb C* mice *Terminalia catappa* extract was administered at a dosage of 80mg/kg. mice were sacrificed by humane decapitation, and their blood collected in EDTA and PCV was determined by benzidine spectrophotometric assay method as previously described by Khalid *et al.* (2003) $P < 0.05$ for both folic acid and *T. catappa*

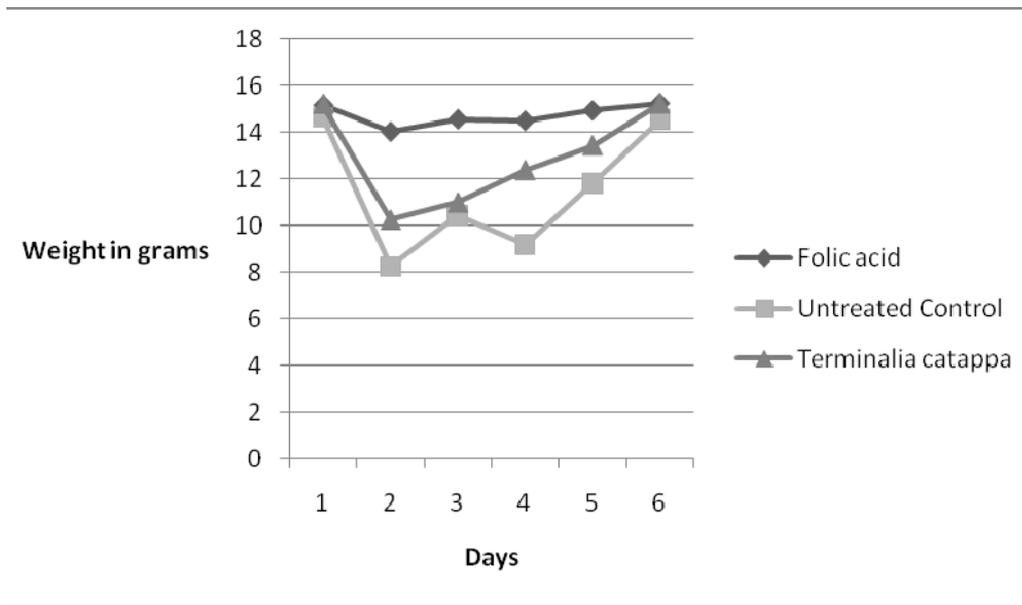


Figure 3. Effect of *Terminalia catappa* extract on the mean weight of adult *Balb C* mice: weight was measured using a Mettler top loading balance at every 24 hours interval for 7 days

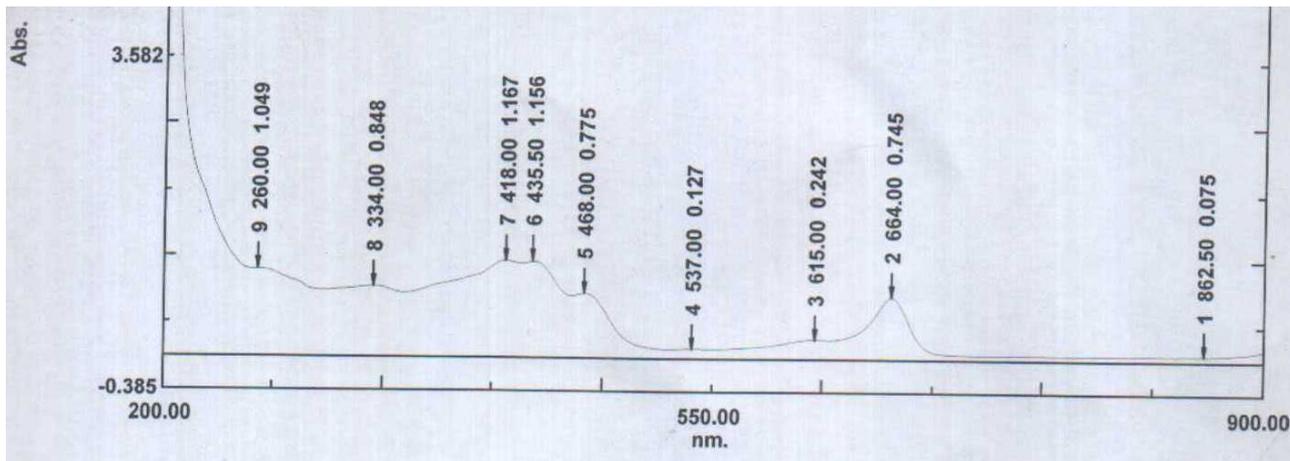


Figure 4. UV-Visible absorbance spectra of *Terminalia catappa* methanolic extract in PBS buffer pH 7.2 path length of 1cm in a quartz cuvette