Original Research Article

Anti-Anemic activity of *Hydro-alcoholic extract* of *Calotropis procera* flower on phenylhydrazine- induced anaemic rats

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Abstract

The present study was to evaluate the anti-anaemic activity of *Calotropis procera* flower of Apocynaceae family. Anemia was induced in rats by intra-peritoneal administration of phenylhydrazine at the dose of 40 mg / kg / day for two days. Hydroalcoholic extract was given orally to anaemic rats at 100 mg/kg and 200 mg / kg body weight, once a day for 28 days. Blood samples were collected from the rats by tail incision on days D0, D2, to the 1st, 2nd, 3rd and 4th week of treatment and subjected to the analysis of red blood cells (RBC), hemoglobin (Hb) and hematocrit (PCV). Extracts at dose of 100mg/kg and as well 200 mg / kg increased ($p < 0.001$) significantly the number of red blood cells in the 4th week of treatment when compared with that untreated anaemic group. In addition, the hemoglobin level increased ($p<0.01$) significantly in the first week of treatment to the rats of groups III and IV which received respectively reference antianaemic (Vitamin B12). The anti-anemic effect of Hydroalcholic aqueous extract was comparable to that of the drug Vitamin B12.

Keywords: *Calotropis procera*, Anaemia, Hydroalcoholic, Hematological Parameters

Introduction

Anaemia is one of the public health problems most widespread, especially in developing countries. It is characterized by the deficiency of red blood cells (RBC) or hemoglobin in the blood, which results in the disturbance of the oxygen transport. The normal rate of hemoglobin varies with age and gender. There is anaemia when the rate is less than 110 g / L for pregnant women and children of 6 months to 5 years, 120 g / L for unpregnant women and 130 g / L for men.(1) There are two groups of anaemia, the lack of production of red blood cells (iron deficiency, aplastic or megaloblastic anaemia) or the abnormal destruction of red blood cells (hemolytic anaemia, or anaemia caused by a chronic disease). Iron deficiency anaemia is the most common type of anaemia. It is most widespread to children and women of all ages. The World Health Organization estimates that for the entire world, anaemia reached a staggering 2 billion people affected, also about 50% of cases is due to iron deficiency.(1) In Côte d'Ivoire, about 80% of children aged 2 to 5 years, 50% of school-age children and 50% of adult women are prone to iron deficiency problems.(2) In the case of hemolytic anemia, the rate of production of red blood cells is normal or high, but they are destroyed too rapidly. This disease is acquired or inherited. Acquired, it may be due to a reaction of the immune system (autoimmune or allergic), in the presence of toxic substances in the blood (phenylhydrazine) or to the infections. Infectious diseases especially malaria, helminthes infections, but also tuberculosis and HIV / AIDS contribute significantly to the elevated figures of prevalence of anaemia that is observed in many places.(2,3) Anaemia is characterized by a large number of symptoms that are losing weight and / or appetite, pallor (skin and complexion), fatigue or unexplained drowsiness, weakness, loss of energy, shortness of breath and many others.

Calotropis procera (Asclepiadaceae), a giant milk weed, is known for its pharmacological importance for centuries. The coarse shrub is a very promising source of anticancerous, ascaricidal, schizonticidal, anti-microbial, anthelmintic, insecticidal, anti-inflammatory, anti-diarrhoeal, larvicidal with many other beneficial properties. Plant is described as a golden gift for human kind containing calotropin, calotropagenin, calotoxin, calactin, uscharin, amyrin, amyrin esters, uscharidin, coroglaucigenin, frugoside, corotoxigenin, calotropagenin and voruscharine used in many therapeutic applications. Different compounds like norditerpenic esters, organic carbonates, the cysteine protease procerain, alkaloids, flavonoids, sterols and numerous cardenolides made this plant of scientific attraction for centuries. Plant is not only a great source of natural hydrocarbons but also contains several metabolites used as folk medicine for the treatment of leprosy, elephantiasis, fever, menorrhagia, malaria and snake bite.

Materials and Methods

Plant material: The plant material is made up of the flower of *Calotropis procera*. The flower was collected from medicinal garden of Modern institute of Pharmaceutical Sciences in August 2016. The sample of plant was identified and authenticated at Rajmata Vijayraje Scindia Krishi Vishwavidhalaya, College of Agriculture, Indore

Preparation of extracts: The flower of *Calotropis procera* was cut up, shade dried under room temperature for a period of three weeks. The dried plant material was made to a coarse powder and 100g of flower was extracted in Soxhlet assembly using 70:30 ratios of ethanol and water respectively

Phytochemical screening: The different groups of compounds (sterols, polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones, saponins and cardiac

glycosides) have been researched in the extracts of C.procera according to the standard procedures.[12-15]

Experimental animals: Male Wistar rats (35) of weighing between 150-230 g were used for this study. The animals were housed in plastical cages and acclimatized for two week in the animal house of the MIPS, Indore. They had been maintained under standard conditions (room temperature 25°C ± 3°C, humidity 35to 60%, light and dark period 12/12 hours). All animals had regular supply of clean drinking water and food.

Induction of anaemia: Anaemia was induced in rats by intraperitoneal administration of 40 mg / kg / day of phenylhydrazine (PHZ) for two days (D0 and D1).[16,17] The treated rats with phenylhydrazine whose haemoglobin concentration <13 g / dl were considered as anemic and included for the study.

Treatment of animals: Five groups of 5 rats were formed and treated daily for 4 weeks as follows:

- Group I (G1) - Normal control received 10 ml / kg of 0.5% CMC (carboxy methyl cellulose) from day D2 to D28.
- Group II (G2) -Anaemic control received 0.5% CMC (10 ml / kg) from day D2 to D28.
- Group III (G3) - Treated with Vitamin B12 (Vit B12) syrup (1 ml /day) from day D2 to D28.
- Group IV (G4) - Treated with Hydroalcholic extract of C.procera (100 mg / kg) from day D2 to D28.
- Group V (G5) - Treated with Hydroalcholic extract of C.procera (200 mg / kg) from day D2 to D28.

All administration was done orally using oropharyngeal cannula once per day for 28 days (4 weeks).

Analysis of haematological parameters: Blood samples were collected from the rats by tail incision before induction of anaemia (D0), after induction of anemia with PHZ (D2) and at 1st, 2nd, 3rd and 4th weeks of treatment.[18,19] The red blood cell number (RBC), haemoglobin concentration (Hb) and haematocrit were determined at days D0, D2, D7, D14, D21 and D28 using an automatic blood cell counter (Sysmex KX 21) and the variations of average values of hematological parameters were calculated relative to the mean values of D0 and D2.

Statistical analysis: Graph Pad Prism 5.0 software (Microsoft, USA) was used for the analysis of the results obtained. The mean value is accompanied by the standard error of mean (mean ± SEM). It was taken to the ANOVA (one way ANOVA followed Dunnet's Test) test to verify the normality of variables. The significance level was set at $p < 0.05$

Results

Phytochemical analysis of Hydroalcholic extracts of C.procera flower revealed the presence of large chemical groups that are: alkaloids, polyphenols, sterols, terpenes, catechin tannins, flavonoids, leucoanthocyanins, quinones, saponins and cardiac glycosides [Table 1].

Table 1: Various chemical compounds identified in C.procera flower

Chemical Groups	Hydroalcholic Extract
Alkaloids	+ve
Polyphenols	+ve
Sterols and Terpenes	+ve
Catechin Tannins	-ve
Gallic tannins	-ve
Flavonoids	+ve
Quinones	+ve
Cardiac Glycosides	-ve
Saponins	+ve
Leucoanthocyanins	-ve

Effect of aqueous and hydroalcoholic extracts of C.procera flower on haematologicalparameters

Red blood cells: After injection of phenylhydrazine to rats of the six groups except the normal group, there was a decrease in red blood cells (47.95% ± 2.21) at day D2. An increased number of red blood cells was observed after treatment in the following days. The results show that the rats of the groups G3, G4 and G5 have almost completely recovered at the 4th week (94.66%, 98.33% and 92.85% recovery respectively).

Haemoglobin: The administration of phenylhydrazine at day D2 caused a significant decrease ($p < 0.01$) haemoglobin rate in rats of G2, G3, G4, and G5 of 42.86% ± 1.69. After treatment, a progressive recovery is obtained on the following days [Table 4]. The results show in one hand that the rats that received Vitamin B12 and those which received extract aqueous of C.procera have almost completely recovered at the 4th week ($p < 0.001$), and at the other hand, that the Hydroalcholic Extract at dose of 200 mg / kg /day allows a faster recovery.

Haematocrit: The administration of phenylhydrazine also decreased hematocrit at day D2. This decrease is 17.58%, 20.28%, 19.70%, 19.03, 21.36 and 17.77 respectively in untreated rats G2, the rats of groups G3, G4, and G5. After treatment the increased of hematocrit at day D7 (1st week) was 41.17% in untreated anaemic rats, 41.93%, 40.75%, and 41.60%, respectively in the rats of groups G3, G4, and G5. By the fourth week (D28), % in rats of group G2.

Table 2: Effect of Hydroalcholic extracts of *Calotropis procera* flower on the number of red blood cells during and after induction of anaemia with phenylhydrazine in rats

Drug treatment	RBC (106cells/ /µl)					
	D0	D2	1st week	2nd week	3rd week	4th week
Normal Control (10 ml/kg of 0.5% CMC)	7.08 ± 0.07	7.40±0.10	7.46 ± 0.24	7.39±0.04	6.46±0.19	7.14±0.26
Anaemic Control (10 ml/kg of 0.5% CMC)	7.33 ± 0.15	4.32 ± 0.16 −41.06[a**]	5.77 ± 0.10 +33.56[b]	6.69±0.15 +54.86[b]	6.97±0.07 +61.34[b]	7.33±0.27 +69.67[b**]
Vit B12 syrup (1ml/day)	7.51 ± 0.12	3.75 ± 0.14 −50.06[a**]	5.02 ± 0.17 +**33.86**[b]	5.92±0.16 +**57.86**[b]	6.42±0.14 +**71.20**[b]	7.30±0.27 +**94.66**[b***]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D2	7.65 ± 0.06	3.64 ± 0.25 −52.41[a**]	5.23 ± 0.21 +43.68[b]	5.90±0.36 +62.08[b]	6.09±0.14 +67.30[b]	7.02±0.01 +92.85[b***]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D0	7.43 ± 0.14	4.38 ± 0.10 −41.04[a**]	5.53 ± 0.18 +26.25[b]	+26.25 +26.25[b]	χ+63.69[b*]	7.52±0.08 +71.68[b**]

Table 3: Effect of hydroalcholic extract of *Calotropis procera* flower on the Hemoglobin during and after induction of anemia with phenyl hydrazine in rats

Drug treatment	Haemoglobin (g/dl)					
	D0	D2	1st week	2nd week	3rd week	4th week
Normal Control (10 ml/kg of 0.5% CMC)	13.10 ± 0.11	13.25± 0.15	13.37± 0.17	13.20± 0.40	13.20±0.40	12.3±0.40
Anaemic Control (10 ml/kg of 0.5% CMC)	13.18 ± 0.10	8.3 ± 0.10 −37.02[a**]	8.33±0.70 48.55[b]	8.80± 0.10 +54.21[b]	8.10±0.40 +57.83[b*]	8.10±0.40 +61.44 [b**]
Vit B12 syrup (1ml/day)	13.78 ± 0.14	7.75 ± 0.15 −43.75[a**]	13.00±0.24 +67.74[b**]	13.40± 0.210 +72.90[b**]	13.47±0.08 +73.80[b***]	13.63±0.08 +75.87[b***]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D2	13.82 ± 0.10	7.66 ± 0.14 −44.57[a***]	12.60± 0.05 +64.49[b*]	12.93± 0.23 +68.79[b**]	13.±0.30 +69.71[b**]	13.25±0.25 +72.97 [b**]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D0	13.3 ± 0.37	8.16 ± 0.03 −38.64[a**]	12.70 ± 0.49 +55.63[b]	13.70±0.05 +67.89[b**]	13.70±0.35 +67.89[b**]	13.97±0.08 +71.20[b***]

Table 4: Effect of Hydroalcholic Extracts of *Calotropis procera* flower on Haematocrit during and after induction of anaemia with phenyl hydrazine in rats

Drug treatment	Haematocrit (%)					
	D0	D2	1st week	2nd week	3rd week	4th week
Normal Control (10 ml/kg of 0.5% CMC)	42.83 ± 0.24	43.23±0.54	43.65± 0.06	43.97±0.13	42.13±0.03	42.95±0.18
Anaemic Control (10 ml/kg of 0.5% CMC)	42.55 ± 0.06	24.97 ± 0.27 −41.31[a***]	21.17± 0.33 +64.87[b***]	21.84± 0.25 +67.56[b***]	22.25±0.23 +69.20[b***]	22.88±0.15 +71.72[b***]
Vit B12 syrup (1ml/day)	45.16 ± 0.19	24.88 ± 0.27 −44.90[a***]	41.93± 0.22 +68.52[b***]	42.35± 0.15 +70.21[b***]	44.05±0.16 +77.04[b***]	45.17±0.14 +81.55[b***]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D2	43.40 ± 0.18	24.37 ± 0.08 −43.84[a***]	41.60±0.43 +70.70[b***]	42.00± 0.03 +72.34[b***]	42.67±0.21 +75.09[b***]	43.10±0.11 +76.85[b***]
Hydroalcholic Extract of *C.procera* (100mg/kg) from D0	42.90 ± 0.18	25.13 ± 0.29 −41.42[a***]	41.47± 0.18 +65.02[b***]	42.35± 0.12 +68.52[b***]	42.90±0.20 +70.71[b***]	43.68±0.17 +73.81[b***]

Discussion

Plants are a rich source of drugs because they produce a host of bioactive molecules, most likely acts as chemical defense against predators or infectious agents.[20] Phytochemical analysis revealed the presence of large chemical groups that are: alkaloids, tannins, flavonoids, polyphenols, quinones, sterols, terpenes, cardiac glycosides, saponins and leucoanthocyanins. They have antioxidant power, promote regeneration of tissue, reduce the permeability of blood capillaries and increase their resistance to hemolysis.[21] The presence of these chemicals by their properties justifies the resistance of red blood cells of treated rats with the extract. Indeed saponins and alkaloids have shown anti-anaemic properties.[22] Alkaloid inhibits cyclic adenosine monophosphate (cAMP) phosphodiesterase thereby accumulating cAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins, which improves erythropoiesis.[23] Saponins are also known to inhibit platelet aggregation and thrombosis. Saponin containing in herbs have been successfully used in the management of liver inflammation, as tonic sedative formulas, to promote and vitalize blood circulation.[24] Since saponins are active agents which lyse the membrane of red blood cells or other wall, it is likely that red blood cells were first lysed by the plant. Then the cells have overcome this inhibition by producing a glycosidic enzyme which cleaves some of the terminal sugars from the saponin, which causes its detoxification.[24] This detoxification of saponins has reinforced the proper use of iron contained in the aqueous extract of Calotropis procera flower allowing to synthesize heme / haemoglobin for new red blood cells, thus leading to an improvement of Hb, RBC and PCV. Saponins especially terpene glycosides enhance the natural resistance and have the recovery powers of body.[24] Also, flavonoids have anti-anaemic potential and veinotonic properties, which protects the blood capillaries.[21] The anti-anemia potential and haemoglobin restoring effect of aqueous extract of Calotropis procera flower as suggested by the data in this study could be attributed in part to its phytochemical constituents.

As regards the weight of the rats, there was a reduction in body weight after induction of anaemia by phenylhydrazine [Table 2]. This observation is in agreement with the previous report of Saimak.[24] The loss of body weight is one of the symptoms of anaemia, this would be due to lack of appetite in anaemic rats. During treatment these rats resumed appetite thus promoting body weight gain. This decrease in body weight in anemic rats could be explained by a reduction of the activities of disaccharidases (enzymes that catalyze the last stage of carbohydrate digestion) in anemic rats.[24] The aqueous extract of Calotropis procera flower has better improved the percentage of weight gain in treated rats compared to that of rats that received the hydroalcoholic extract at the end of the study period. This improvement in the percentage of weight gain of rats treated with the aqueous extract is in line with that of the anti-anemic rats that received antianemic of reference Vit B12.

The intra peritoneal administration of 40 mg / kg / day of phenylhdrazine for 2 days (D0 and D1) in Wistar rats caused a significant mean decrease of the concentration of hemoglobin, red blood cells and the packed cell volume (PCV). The rats of groups IV and V received the extracts of the plant at the same time as the phenylhdrazine administration (from day D0). The treatment from day D0 allowed red blood cells from the beginning to develop a resistance vis a vis of PHZ and the extract containing the saponin. Indeed, in our study, rats that received assigns at the same time the aqueous extract of Calotropis procera flower and PHZ at days D0 and D1 have a Hb concentration, a number of red blood cell and haematocrit more higher than those of the rats of other groups. This increase was progressive throughout the treatment. Considering the results of the groups IV and V, the Hydroalcholic extract of C.procera flower has a higher anti-anaemic. In addition, Vit B12 reference drug showed a significant increase ($P < 0.01$) of the content in haemoglobin after the first week of treatment. The anti-anemic effect of the Hydroalcholic extract of C.procera flower was comparable to that of Vit B12.

Conclusion

The injection of phenylhydrazine to rats caused a hemolytic anemia characterized by reducing hematological parameters. The oral administration of aqueous and ethanol extracts of *Calotropis procera* in the dose of 200 mg / kg / day significantly increased haemoglobin level in the first week of treatment. The anti-anaemic effect of the aqueous extract was more pronounced than that of the hydroalcoholic extract (heamoglobin content 87% against 70% at the fourth week). The anti-anaemic potential of the plant could come from phytochemicals and also the possible vitamin and mineral constituent

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