



EVALUATION OF ANTI-ANEMIC ACTIVITY OF WITHANIA SOMANIFERA ROOT EXTRACT IN QUINIDINE INDUCED ANEMIA IN RATS

RAVIKUMAR BORICHANGAR, KAMAL KALAVADIYA*, KARUNAKAR SHUKLA, SOURABH JAIN

College of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore, M.P., India.

ABSTRACT: Hemolytic anemias are a group of disorders with varied clinical and molecular heterogeneity besides they are categorized by decreased levels of circulating erythrocytes in blood. The most common cause of anemia worldwide is iron deficiency. Iron is involved in the hemoglobin synthesis and indirectly helps in the erythropoiesis by helping in transportation of iron in its soluble form. The speedy and progressive recovery of anemic rats responding to treatment of *Withania Somanifera root extract* may be due to increased erythropoiesis and protection against hemolysis by oxidative stress. These results support the ethnobotanical use of *Withania Somanifera root extract* in the treatment of anemia.

Keywords: Anemia, Hemolytic, *Withania Somanifera*, Root extract, Iron.

I. INTRODUCTION:

Anemia is well-defined as hemoglobin lower two standard deviations of the mean for the age and gender of the patient. Iron is a crucial element of the hemoglobin molecule. The most common cause of anemia worldwide is iron deficiency, which results in microcytic and hypochromic red cells on the peripheral smear¹. Hemolytic anemias are a group of disorders with varied clinical and molecular heterogeneity besides they are categorized by decreased levels of circulating erythrocytes in blood². Infections with SARS-CoV-2 can result in severe clinical manifestations. patients present with systemic inflammation, studied reported that the prevalence and predictive value of anemia of inflammation or functional iron deficiency, originating from immune-mediated alterations of iron homeostasis³.

High prevalence of co-morbid anemia and overweight, as well as comorbid anemia and diabetes, indicating that the double burden of malnutrition is now a severe public health concern in rural regions of South India⁴. Anemia is defined as a lower than normal number of red blood cells (RBCs) in the blood, usually measured by a decrease in the amount of hemoglobin. Hemoglobin is an iron-rich protein in RBCs that transports oxygen to the body's muscles and organs. With anemia, the body produces too few healthy RBCs, loses them, or destroys them⁵.

Low level of folate, zinc, and vitamin A were associated with a high level of anemia among children besides most of the studies in India related to anemia among children are related to children aged 6–59 months⁶. Anemia is significantly higher among younger women (15–24 years), women with primary or no education, women from the poorest wealth quintile, women without toilet facilities and improved water sources, underweight women, and women with more than one children born in last five years in most of the countries⁷. Sample of US children, we found a significant and positive association between anemia and neurodevelopmental disorders⁸. Overall prevalence of anemia and moderate-severe anemia of 16.8% and 5%, respectively, among Ecuadorian non-pregnant women of reproductive age. This percentage positions anemia as a mild public health concern. Additionally, it explored a set of demographic, socio-economic and reproductive factors associated with anemia and moderate-severe anemia⁹.

Ayurvedic formulations based on Ashwagandha considered the best and safer alternatives for synthetic drugs¹⁰. Ashwagandha chemical analysis showed that the presence of functional groups like phytochemical

constituents^{11,12}. *Withania somnifera* root powder on general performance and hematological profile against fenvalerate induced toxicity in cockerels besides concluded that supplementation of Ashwagandha root powder through feed may reduce the severity of fenvalerate toxicity in cockerels¹². Studied showed that the Ashwagandha root powder was helpful in curing the infection, overcoming diabetes and anemia¹³. *Withania Somanifera root extract* shows significant pharmacological activity due to presence wide range of active principles which includes coumestans, alkaloids, flavonoids, glycosids, polyacetylenes, and triterpenoides¹⁶.

The effect of quinine and quinidine introduction in subtoxic doses on the characteristics of the hemopoietic and lymphoid organs was studied in BALB/c mice. The treatment led to reversible anemia and neutrophile leukocytosis in peripheral blood. The quinine-induced decrease in erythrocytes was accompanied by reticulosis, lowered erythrocyte osmoresistance, and intensified spleen erythropoiesis; quinidine induced reticulocytopenia and a decrease in the bone marrow erythroblasts¹⁴. Drug induced hemolytic anemia as seen after exposure of quinidine in rats and monkey.

II. MATERIALS AND METHODS:

2.1 Plant collection

Dry plant of *Withania Somanifera root extract* TAB were collected from Medica Pharma Junagadh, Gujarat, India.

2.2 Authentication

Authentication of *Withania Somanifera* plant was done at Himalaya Drug company.

2.3 Preparation of extract

The plant was collected, dried then grounded into coarse powder. Powder was than complete extraction by maceration method using 70% ethyl alcohol as a solvent. Hydro alcoholic extract concentrated by vacuum distillation to dry. Extract was stored in desiccator.

2.4 Fractionation of hydro alcoholic extract of *Withania Somanifera root extract*

Fractionation process was done by using four different solvent chloroform, ethyl acetate, acetone, and water.

2.5 In vivo

Albino wistar rats (180-200g) of either sex were used. They were housed at ambient temperature ($22\pm 10^{\circ}\text{C}$), relative humidity ($55\pm 5\%$) and 12h/12h light dark cycle and provide pelleted diet and purified drinking water. The protocol of the experiment is approved by the Institutional Animal Ethical Committee (IAEC) as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.6 Study design for acute toxicity study

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Anemia was induced by intraperitoneal injection of phenyl hydrazine hydrochloride (QUINIDINE) at 40mg/kg for 2 days. The dose of WS-HAWS was selected from results of acute toxicity study.

2.7 Evaluation of parameters

Hematological parameters Hb, RBC, WBC, Hematocrit and Platelet count were estimated on day 0, 3, 7, and 15. Blood was collected by retro orbital puncture then serum was separated by centrifugation and was used for estimation of hematological parameters. Change of body weight in grams of each animal was recorded at day 0, 3, 7, and 15.

III. RESULTS

3.1 Ethanolic extract of plant % Yield The percentage yield of ethanolic extract of *Withania Somanifera root extract* was 14.0% W/W.

3.2 Effect of hydro alcoholic extract of *Withania Somanifera root extract* on induced hemolysis in RBC.

Sr. no.	Fraction	% Yield
1	Chloroform	2.25% W/W
2	Ethyl acetate	2.15%W/W
3	Acetone	3.17% W/W
4	Water	3.07% W/W

Table 1 % yield of fractions of hydro alcoholic extract.

Sr no.	Con.mg/ml	% Hemolysis	
		Extract of <i>Withania Somanifera root extract</i>	Gallic acid
1	50	26.59±0.72	25.68±1.89
2	25	54.44±1.10	38.52±1.80
3	12.5	77.80±1.10	69.05±1.12
4	6.25	88.10±0.72	89.72±1.73
5	3.1	94.42±0.86	91.17±0.72
6	1.55	91.13±0.41	94.15±0.42

Table 2 In vitro Anti hemolytic activity of hydro alcoholic extract of *Withania Somanifera root extract*

3.3 Fractionation of hydro alcoholic extract of *Withania Somanifera root extract*:

3.3.1 Effect of various fractionation of hydro alcoholic extract of *Withania Somanifera root extract* on induced hemolysis in RBC

Sr. no	Con. mg/ml	Gallic acid Standard	% Hemolysis			
			Chloroform	Ethyl acetate	Acetone	Water
1	50	25.68±0.60	43.13±0.60	43.97±0.60	26.89±0.39	32.77±0.39
2	25	38.52±1.95	51.82±0.60	52.94±0.79	44.81±0.60	49.01±0.60
3	12.5	69.05±3.52	65.82±0.60	67.78±0.82	59.66±0.39	63.58±0.82
4	6.25	89.72±0.60	74.22±0.60	77.59±1.27	69.74±0.39	63.58±0.82
5	IC50	37.83 mg/ml	10 mg/ml	9.17 mg/ml	14 mg/ml	17.08mg/ml

Table 1 In vitro Anti hemolytic activity of all fractions of hydro alcoholic extract of WS

Results showed that the lower the IC50 the more protection offered against hemolysis by the fraction. The hydro alcoholic extract of *Withania Somanifera* root extract contain ethyl acetate fraction have less IC50 value. This result suggest that ethyl acetate fraction is better anti-hemolytic activity and offered significant biological action compared with standard compound used.

3.3.2 Acute toxicity study of active fraction of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract

To evaluate safety of WS-HAWS(2gm/kg), it was given to animals as per OECD guideline 423 for acute toxicity study and observed for following parameters:

3.3.2.1 Body weight

Acute toxicity study of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract shows that there is no significant change in body weight from day 0 to day 14. WS-HAWS(2gm/kg) showed no significant change in various autonomic and behavioral response of rat compared to normal animals. No sign of toxicity was observed in 24 hr. No mortality was recorded in first 48 hr till 14 days in animal treated with WS-HAWS up to 0.2gm/kg oral dose.

RAT	DAY 0	DAY 7	DAY 14	% Change in body weight
MEAN± SEM	210.00±5.77	212.66±6.36	212.50±2.50	0.11 %

Table 2 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract (2gm/kg)

It is therefore considered to be safe at dose of 2gm/kg.

3.3.2.2 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Hemoglobin content at various dose

DAY	Hemoglobin (g/dl)					
	NC	DC	STD	T1	T2	T3
0	14.02±0.0	14.31±0.1	14.03±0.08	14.22±0.10	12.34±0.71	14.18±0.10
3	14.09±0.0	8.79±0.00	9.10±0.01#	9.09±0.02#	13.10±0.69	9.12±0.03#
7	14.50±0.1	8.22±0.10	11.60±0.17	12.38±0.12	12.57±0.44	12.24±0.03
15	14.60±0.0	7.13±0.01	13.53±0.07	14.24±0.01	13.97±0.33	14.93±0.02
	8	#	*	*	*	*

Table 3 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on hemoglobin content in QUINIDINE –induced anemia.

NC = Normal control, DS= Disease control, Standard, T100- EA-HAE, T200- EA-HAE, T300-EA-HAEA. * Significantly different from normal control (p< 0.05) # Significantly different from disease control (p < 0.05).

On day 15, Hb content significantly increased and normalized (p < 0.05) in all QUINIDINE treated groups (T1, T2, T3, STD) compared to disease control (Group DC). The treatment with WS-HAWS was found to be in a dose dependent

manner with higher dose giving highest effect and is comparable to the treatment with standard (Group STD).

3.3.2.3 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Red blood corpuscles (RBC) at various dose

On day 15, mean total RBC count was significantly increased ($p < 0.05$) and normalized in all QUINIDINE treated group compared to (Group DC). The treatment with WS-HAWS was found to be in a dose dependent manner with higher dose giving highest effect and is comparable to the treatment with standard (Group STD).

RBC Count ($10^{12}/L$)						
DAY	NC	DC	STD	T1	T2	T3
0	7.39±0.00	7.56±0.00	7.56±0.07	7.42±0.00	7.18±0.32	7.50±0.00
3	7.39±0.00	3.84±0.01#	3.85±0.01#	3.78±0.01#	3.90±0.00#	3.82±0.00#
7	7.48±0.00	3.56±0.00#	5.31±0.11	7.24±0.00	7.86±0.00	8.87±0.00
15	7.54±0.00	3.75±0.00#	7.43±0.00	8.14±0.00	8.87±0.00	9.78±0.00
			0*	0*	0*	4*

Table 4 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on RBC in QUINIDINE –induced anemia.

3.3.2.4 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on white blood cells (WBC) at various dose

On day 15, the mean total leukocyte counts of QUINIDINE treated group (T1, T2, T3) were significantly decreased and normalized ($p < 0.05$) comparable to (Group DC). The treatment with WS-HAWS was found to be in a dose dependent manner with higher dose giving highest effect.

WBC Count ($10^9/dl$)						
DAY	NC	DC	STD	T1	T2	T3
0	13.41±0.00	13.42±0.00	13.41±0.01	13.41±0.01	13.44±0.03	13.43±0.02
3	13.76±0.05	23.23±0.04#	23.11±0.00#	23.21±0.02#	23.22±0.02#	23.24±0.01#
7	13.58±0.65	26.40±0.12#	24.23±0.01	18.89±0.02	17.19±0.06*	17.03±0.01*
15	14.84±0.02	28.91±0.01#	25.58±0.09	14.50±0.01*	13.00±0.00*	12.07±0.04*

Table 5 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on WBC in QUINIDINE –induced anemia.

3.3.2.5 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Hematocrit at various dose

On day 15, the hematocrit level significantly increased ($p < 0.05$) and normalized in all QUINIDINE treated group compared to disease control (Group DC). The treatment with WS- HAWS is found to be in a dose dependent manner with highest dose giving highest effect and is comparable to the treatment with standard.

DAY	Hematocrit Count (%)					
	NC	DC	STD	T1	T2	T3
0	42.80 ±1.4	43.74 ±1.37	43.58±1. 47	41.63±1. 19	42.28±1. 03	42.26±0. 81
3	42. 09± 1.5	22.71±0. 47	27.91±0. 25	25.75±0. 26	29.95±0. 38	31.28±0. 42
7		#	#	#	#	#

Table 6 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Hematocrit in QUINIDINE –induced anemia.

3.3.2.6 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Platelets at various dose

On day 15, the platelets level significantly ($p < 0.05$) normalized in QUINIDINE treated group (T1, T2 T3) compared to disease control (Group DC). The treatment with WS-HAWS is found to be in a dose dependent manner with highest dose giving highest effect.

DAY	Platelet count ($10^9/l$)					
	NC	DC	STD	T1	T2	T3
0	307.33± 0.49	320.16±1.8 6	307.00±0.5 1	315.83±2.7 8	306.50±0.7 6	311.50±0.6 1
3	307.50± 0.42	455.50±2.2 6#	463.50±1.33 #	465.16±1.1 6#	465.50±0.9 5#	465.00±0.5 7#
7	308.00± 0.36	470.00±0.3 6#	452.50±1.9 9	423.16±1.7 0*	411.66±1.8 9*	403.16±0.6 0*
15	308.00± 0.36	486.66±1.4 0#	441.33±1.2 2	379.16±0.4 0*	359.16±0.6 0*	348.83±0.4 7*

Table 7 Effect of ethyl acetate hydro alcoholic extract of *Withania Somanifera* root extract on Platelets in QUINIDINE –induced anemia

IV. CONCLUSION

Administration of QUINIDINE also resulted in an increase in WBC, Platelets. MCHC and MCH values were also increased, which may be due to reduced Hb and RBC and increase in the free plasma Hb. This condition is also common in Vitamin B12 and folate deficiencies and has also been withania in rats infected with *Trypanosoma brucei* has been linked to iron deficiency anemia. However, the hematological parameters were restored to normal range after treatment. It was also observed that the recovery of the treated groups was dose dependent with the highest dose of affecting the highest change. The speedy and progressive recovery of anemic rats responding to treatment of WS-HAWS may be due to increased erythropoiesis and protection against hemolysis by oxidative stress. The improvement in the hematological indices exhibited by WS-HAWS might be connected with the Iron content of *Withania Somanifera* root extract. These constituents might have a direct influence on the protection of hemolysis by ROS generated by quinidine. Iron is involved in the hemoglobin synthesis and circuitously helps in the erythropoiesis by helping in transportation of Iron in its soluble form. These consequences provision the ethnobotanical use of *Withania Somanifera* root extract in the treatment of anemia.

V. CONFLICT OF INTEREST

Authors declared they have no conflict of interest.

VI. ACKNOWLEDGMNET

I would like to thank my supervisor Karnunakar Shukla and co-supervisor Saurabh Jain for expert advice and encouragement throughout work.

VII. REFERENCES:

- [1] Warner, M. J., & Kamran, M. T. 2020. Anemia, iron deficiency. *StatPearls [Internet]*.
- [2] Jamwal, M., Sharma, P., & Das, R. 2020. Laboratory approach to hemolytic anemia. *The Indian Journal of Pediatrics*, 87(1), 66-74.
- [3] Bellmann-Weiler, R., Lanser, L., Barket, R., Rangger, L., Schapfl, A., Schaber, M., ... & Weiss, G. 2020. Prevalence and predictive value of anemia and dysregulated iron homeostasis in patients with COVID-19 infection. *Journal of clinical medicine*, 9(8), 2429.
- [4] Little, M., Humphries, S., Dodd, W., Patel, K., & Dewey, C. 2020. Socio-demographic patterning of the individual-level double burden of malnutrition in a rural population in South India: a cross-sectional study. *BMC Public Health*, 20, 1-14.
- [5] Mileti L. 2008 Anemia. In: Loue S.J., Sajatovic M. (eds) *Encyclopedia of Aging and Public Health*. Springer, Boston, MA. 33754-8_34.
- [6] Rahman, M. H. U., Chauhan, S., Patel, R., Boro, B., Anwar, T., Kumar, P., ... & Srivastava, S. 2020. Anaemia among Indian children: A study of prevalence and associated factors among 5-9 years old. *Children and Youth Services Review*, 105529.
- [7] Sunuwar, D. R., Singh, D. R., Chaudhary, N. K., Pradhan, P. M. S., Rai, P., & Tiwari, K. 2020. Prevalence and factors associated with anemia among women of reproductive age in seven South and Southeast Asian countries: Evidence from nationally representative surveys. *PloS one*, 15(8), e0236449.
- [8] Yang, W., Liu, B., Gao, R., Snetselaar, L. G., Strathearn, L., & Bao, W. 2020. Association of Anemia with Neurodevelopmental Disorders in A Nationally Representative Sample of US Children. *The Journal of Pediatrics*.
- [9] Sosa-Moreno, A., Reinoso-González, S., & Mendez, M. A. 2020. Anemia in women of reproductive age in Ecuador: Data from a national survey. *PloS one*, 15(9), e0239585.
- [10] Rasheed, A., Roja, C., Reddy, G. A. K., & Reddy, B. S. 2012. Formulation, standardization and pharmacological evaluation of a poly herbal traditional remedy-Ashwagandharishtam. *Oriental Pharmacy and Experimental Medicine*, 12(1), 51-58.
- [11] Kushwaha, R., & Karanjekar, S. 2011. Standardization of ashwagandharishta formulation by TLC method. *Int J ChemTech Res*, 3, 1033-1036.
- [12] Tikore, P., Rathod, P., Ingole, R. S., Khose, K. K., & Khodke, M. V. 2019. Protective effect of (Ashwagandha) *Withania somnifera* root powder on general performance and hematological profile against fenvalerate induced toxicity in cockerels.
- [13] Singh, S., & Goshwami, N. 2019. Processing and utilization of ashwagandha for preparation of herbal laddu. *Journal of Pharmacognosy and Phytochemistry*, 8(1), 2149-2150.
- [14] Karpova GV, Berchuk TIu, Abramova EV. Vliianie khinina 2001 [Effect of quinine and quinidine on the status of hematopoietic and lymphoid organs]. *Eksp Klin Farmakol*. Nov-Dec;64(6):48-51.
- [15] Poulet, F. M., Penraat, K., Collins, N., Evans, E., Thackaberry, E., Manfra, D., ... & Abutarif, M. 2010. Drug-induced hemolytic anemia and thrombocytopenia associated with alterations of cell membrane lipids and acanthocyte formation. *Toxicologic pathology*, 38(6), 907-922.
- [16] Jaglan BD, Gill R., 2013. Pharmacological Activity and Chemical Constituents of *Withania Somanifera* root extract, *Int J.Res Dev in Pharmacy and Life Sci*” 13(7).