Vol. 10/2 (2010) 163 - 169

JOURNAL OF NATURAL REMEDIES

Haematopoietic activity of the seed aqueous extract of *Hunteria umbellata* (K. schum) hallier f. in experimental anaemia

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Abstract

This study evaluates the haematopoietic and immunostimulatory activity of the seed aqueous extract of *Hunteria umbellata* (K. Schum) Hallier f. (*HU*) in normal and cyclophosphamide-induced pancytopaenic Wistar albino rats for 28 days. Results showed that oral pre-treatment with 50-200 mg/kg/day *HU* cause significant ($p<0.05$, $p<0.01$) dose related increases in haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC) and lymphocyte differential (%lymp) in normal rats. Also, *HU* significantly ($p<0.05$, $p<0.01$, $p<0.001$) attenuated the cytotoxic effect of cyclophosphamide on the measured haematological parameters in the cyclophosphamide-treated rats, with 200 mg/kg/day *HU* offering the greatest protection. Thus, results obtained in this study suggest that *HU* possesses haematopoietic and immunostimulatory activity mediated possibly by promoting haematopoiesis through enhanced DNA synthesis/repair.

Keywords: *Hunteria umbellata* (K. Schum) Hallier f.; haematopoietic and immunostimulatory activity; cyclophosphamide; Wistar rats

1. Introduction

Anaemia constitutes a major public health problem affecting over 2.2 billions individuals worldwide with a higher prevalence in the developing than developed countries [1, 2]. Its prevalence is higher among the younger (particularly children) than the older population in the developing countries and this can be attributed to malnutrition, helminthic infestation, febrile illnesses (such as severe malaria, septicaemia, etc.) and sometimes haemoglo-binopathies (such as sickle cell disease and thalassemia) [3, 4, 5]. Higher

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prevalence of anaemia has equally been reported in the persons 65 years and above [6]. In the young and middle-age adult population, anaemia could result from haemorrhage, pregnancy, severe infestation with intestinal worms and liver flukes, chronic heart, liver and renal diseases, cancers, auto-immune diseases (rheumatoid arthritis, polyarthritis nodosa, systemic lupus erythromatosis, etc.) and occasionally drug related, particularly, with prolonged used of sulphonamide-containing drugs and anticancer drugs [7].

In recent time, drug-induced anaemia has been a source of worry for cancer patients on chemotherapy. The reason being that most anticancer drugs, particularly, the alkylating agents, have non-selective cytotoxicity to normal body cells, particularly tissues with high mitotic indices, causing deleterious effect on these tissues. Also, despite the success recorded with the use of convectional management including haematinics, blood transfusion, bone marrow transplant, and genetically engineered haematopoetin, etc., the rural dwellers and the urban poor continue to depend on alternative or complementary medicine for their treatments [2]. In the world today, there is a revolution in health care system, resulting in greater acceptance and use of herbal medicine. According to the World Health Organization, about 80% of the world's population depends wholly or partly on plant-derived pharmaceuticals [8]. This is because the exorbitant cost and associated intolerable side-effects of most conventional pharmaceuticals prevent most people from being able to acquire them [9]. In this same vein, some anaemic patients resort to the use of alternative or complementary therapies such as vitamin supplements or herbal remedies either alone or as adjunct to their synthetic drugs. One of such herbal remedies is the cold decoction made from the dried seeds of *Hunteria umbellata* (K. Schum) Hallier f.

Hunteria umbellata (K. Schum) Hallier f. which closely resembles *Picralima umbellata* K. Schum, *Polyadoa umbellata* (K. Schum) Stapf., *Hunteria elliotii* (Stapf) Pichon, *Picralima nitida*, *Picralima elliotii* (Stapf) Stapf., and *Picralima gracilis* A. Chev., belongs to the family Apocynaceae. Among Yoruba and Benin herbalists (Southwest Nigeria), the dried seeds of *Hunteria umbellata* (K. Schum) Hallier f. are locally used for the treatment of yaws and other sexually transmitted infections, stomach ache and ulcers, diabetes mellitus and dysmenorrhoea [10, 11]. Recent ethnobotanical survey conducted by us among Yoruba herbalists (Southwest Nigeria) revealed that plant seeds are also highly valued in the local treatment of obesity, hypertension, pain and swellings, anaemia and as immune booster. Recently, we reported the oral hypoglycaemic effect of the seed aqueous extract of *Hunteria umbellata* (K. Schum) Hallier f. in normal and experimental models of diabetes mellitus which were mediated via adrenergic inhibition and intestinal glucose uptake inhibition [12] and improvement in insulin resistance [13]. However, as part of our efforts in screening for the therapeutic potentials of the plant, the present study was designed to investigate the haematological effect of 28 days of oral treatment with 50-200 mg/kg *HU* in normal and cyclophophamide-treated rats. The choice of the chosen dose range was based on the result of orientation study earlier conducted.

2. Material and methods

2.1. Plant material and its extraction

Two hundred grams (200 g) of dried seeds of *Hunteria umbellata* (K. Schum) Hallier f. were purchased from a retailer of herbal produce in Jakande Estate, Oke-Afa, Isolo, Lagos State,

Table 1. Haematopoietic and immunostimulatory activities of 50-200 mg/kg *HU* in normal rats treated for 28 days

Treatment group	RBC (x106/µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCHC (g/dl)	PLT (x103/µl)	TLC (x103/µl)	% lymph
I	7.0 ± 0.5	14.7 ± 0.4	44.3 ± 1.9	61.9 ± 0.6	28.3 ± 2.8	625.7 ± 44.2	9.0 ± 1.3	77.7 ± 2.2
II	7.2 ± 0.3	14.9 ± 0.4	44.6 ± 1.1	62.1 ± 1.2	29.1 ± 2.0	637.5 ± 29.3	12.6 ± 3.1	85.2 ± 1.3
III	8.1 ± 0.2	15.9 ± 0.5	47.4 ± 1.7	64.2 ± 0.6	32.1 ± 0.3	651.5 ± 55.1	13.7 ± 2.1	88.6 ± 0.7
IV	9.9 ± 0.3	17.4 ± 0.5a	49.9 ± 1.4a	65.4 ± 0.6	32.0 ± 0.1	682.5 ± 58.8a	19.4 ± 1.8a	92.6 ± 2.5b

a and b represent significant increases at $p<0.05$ and $p<0.001$, respectively, when compared with Group I values while e represents a significant decrease at $p<0.01$ when compared to Group I values. Group I = 10 ml/kg/day distilled water; Group II = 50 mg/kg/day *HU*; Group III = 100 mg/kg/day *HU*; Group IV = 200 mg/kg/day *HU*.

Table 2. Haematopoietic and immunostimulatory effects of 50-200 mg/kg/day *HU* in 30 mg/kg/day cyclophosphamide (CYCLO)-intraperitoneal injected rats

	I	II	III	IV	V
Haematological parameters 25th day post-treatment with *HU*					
RBC (x 106/µl)	07.0 ± 0.2	06.2 ± 0.5	07.5 ± 0.2	06.6 ± 0.5	08.1 ± 0.4
Hb (g/dl)	13.0 ± 0.4	11.6 ± 1.0	13.8 ± 0.5	12.1 ± 0.7	15.2 ± 0.4[a]
PCV (%)	41.9 ± 1.4	38.6 ± 1.8	43.9 ± 1.9	38.3 ± 2.1	47.9 ± 2.3[a]
PLT (x103/µl)	609.4 ± 61.3	640.2 ± 37.7	685.4 ± 23.7	640.4 ± 52.7	764.3 ± 26.1[a]
MCV (fl)	59.9 ± 1.7	63.2 ± 2.7	59.0 ± 1.4	60.2 ± 1.6	58.7 ± 0.7
MCHC (g/dl)	31.0 ± 0.3	30.4 ± 2.0	31.5 ± 0.3	31.8 ± 0.9	32.0 ± 1.1
TLC (x103/µl)	15.8 ± 1.8	11.8 ± 2.1	11.1 ± 2.0	16.0 ± 1.5	18.5 ± 0.5[a]
lymp (%)	69.7 ± 6.5	68.8 ± 7.4	77.8 ± 5.7	82.2 ± 4.7	90.6 ± 1.7[a]
Haematological parameters in CYCLO-treated mice 29th day post-treatment with *HU*					
RBC (x 106/µl)	06.7 ± 0.3	04.3 ± 0.7[d]	06.5 ± 0.2	06.5 ± 0.3	07.7 ± 0.3[a]
Hb (g/dl)	13.7 ± 0.4	08.3 ± 1.2[d]	13.0 ± 0.4	12.9 ± 0.5	14.6 ± 0.5[a]
PCV (%)	40.8 ± 2.3	28.8 ± 2.8[d]	40.1 ± 1.3	39.5 ± 1.4	45.3 ± 1.4[a]
PLT (x103/µl)	601.4 ± 60.6	469.8 ± 47.5[e]	512.0 ± 39.5[d]	515.8±27.8[d]	605.8±42.2
MCV (fl)	63.0 ± 1.3	67.1 ± 3.4	61.1 ± 0.7	61.6 ± 3.7	58.7 ± 0.6
MCHC (g/dl)	33.9 ± 1.7	27.2 ± 4.0	32.0 ± 0.2	32.7 ± 0.5	32.1 ± 0.4
TLC (x103/µl)	10.4 ± 1.3	02.9 ± 0.2[f]	04.3 ± 0.1[e]	05.7 ± 0.8[e]	06.8 ± 0.7[d]
lymp (%)	76.6 ± 0.9	59.0 ± 2.1[d]	78.5 ± 4.2	81.6 ± 3.4[b]	87.4 ± 3.9[b]

a and b represent significant increases at $p<0.05$ and $p<0.01$ while d, e and f represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to Group I values. Group I = 10 ml/kg/day distilled water; Group II = 10 ml/kg/day distilled water + CYCLO; Group III = 50 mg/kg/day *HU* + CYCLO; Group IV = 100 mg/kg/day *HU* + CYCLO; Group V = 200 mg/kg/day *HU* + CYCLO

Nigeria in the February 2009. The plant seeds were identified, authenticated and processed by method earlier described by Adeneye and Adeyemi [12].

A hundred grams (100 g) of the dried seeds was pulverized to white-to-light brown fine powder using domestic blender. 30 g of the fine powdered sample was dissolved in 500 ml of distilled water in a 1 litre Pyrex beaker and was left to stand in the refrigerator at 4ºC for 72 hr, after which the homogenate was then intermittently but rigorously shaken for 6 hr before it was rapidly filtered through a piece of clean white cloth. The filtrate was then transferred to an aerated oven preset at 40ºC and completely dried. The residue, thus obtained, was stored in air-and moisture-tight container which was kept in a refrigerator maintained at -4ºC. From this, a fresh stock was reconstituted in distilled water at a concentration of 100 mg/ml (pH = 4.96), whenever needed.

2.2. Experimental animals and their care

Young adult white albino rats (8-14 weeks and weighing 100-140 g) of either sex used in this study were obtained from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria. The rats were handled in accordance with international principles guiding the Use and Handling of experimental animals [14]. The rats were maintained on standard rat feed (Ladokun Feeds, Ibadan, Nigeria) and water which were made available ad libitum. The rats were maintained at an ambient temperature between 28-30ºC, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 hr of light (06:30 hr-18:30 hr) alternating with approximately 12 hr of darkness (18:30 hr - 06:30 hr). In each experimental model, Wistar rats were randomly divided into groups of 5 rats per group such that the weight difference between and within groups is not more than ± 20% of the average weight of rats in the batch.

2.3. Oral administration of HU to normal rats

In this model, 24 young adult male Wistar rats were randomly allotted to 4 groups of 6 rats per group. Group 1 served as the untreated control and were orally administered with 10 ml/kg body weight/day of distilled water. Group 2-4 rats were orally treated with 50, 100 and 200 mg/kg body weight/day of HU for 28 days. At the end of the treatment period, the rats were anaesthetized with inhaled diethyl ether and blood samples were obtained directly from the heart by cardiac puncture. The blood samples were collected into heparinized sample bottles and analyzed for complete haematological profile.

2.4. Induction of pancytopenia and oral treatment with HU

In this model of drug-induced pancytopenia, 50 young adult Wistar rats (weighing 110-140 g) of both sexes were randomly divided into 5 groups of 10 rats per group. Group 1 and 2 rats served as the untreated control and model control, respectively, and were treated with 10 ml/kg body weight/day of distilled water. Group 3–5 rats were pre-treated with single, daily oral doses of 50, 100 and 200 mg/kg body weight/day of HU, respectively, for 28 days. In addition, rats in Groups 2–5 were administered with intraperitoneal injection of 30 mg/kg body weight/day of cyclophosphamide (Cycram®, Korea United Pharm. Inc. Joendong-Myeon Yeongi-Kun, Chungnam, Korea) between the 25th and 28th day of the study, respectively, using the method of Manjrekar *et al*. [15] but the duration of extract pre-treatment was modified from 14 days to 28 days.

Prior to animal sacrifice on the 25th and 29th day of the experiment, respectively, the rats

were fasted overnight but distilled water was made available ad libitum. Five rats from each treatment and control groups were randomly selected and sacrificed, respectively, in order to obtain blood samples for the full blood count.

2.5. Measurement of haematological parameters in normal and cyclophosphamide-treated rats

Blood samples were collected by cardiac puncture under diethyl ether anaesthesia, using 21 gauge (21G) needles mounted on a 5 ml syringe (Unique Pharmaceuticals, Sango-Otta, Ogun State, Nigeria) into EDTA-coated sample bottles (BD Vacutainer®, BD-Plymouth, Plymouth, U.K.) for full blood count (FBC), which included red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PL), total leucocyte count (TLC) and differential leucocyte count (DLC). The collected blood samples were analyzed using Automated Haematology System (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan).

2.6. Statistical Analysis

Results were presented as mean ± S.E.M of six observations and five observations in normal and cyclophosphamide-treated rats, respectively. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-Newman-Keuls test on SYSTAT 10.6. Statistical significance were considered at $p<0.05$, $p<0.01$, and $p<0.001$.

3. Results

3.1. Calculation of %Yield

Cold extraction of the *Hunteria umbellata* seeds after it was completely dried yielded a deep brown, aromatic solid residue which was soluble in distilled water (pH = 6.9), 99.8% methanol (molecular weight = 32.04, acidity = 0.001% and alkalinity = 0.0002%), "absolute" ethanol (molecular weight = 46.07, acidity = 0.006% and alkalinity = 0.0001). The weight of the solid residue left behind was 23 g, giving a yield of 76.67% (w/w).

3.2. Effect of 28 days of oral HU treatment on the haematological parameters of normal rats

Table 1 shows the effect of repeated daily oral treatment with HU on the red blood cell count (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), total leucocyte count (TLC) and lymphocyte differentials (%lymph) in normal and cyclophosphamide-treated rats. Oral treatment with 50-200 mg/kg HU for 28 days caused significant ($p<0.05$) increase in Hb, PCV, PLT, TLC and %lymphocyte differentials while causing non-significant increase in RBC, MCHC and MCV values in the normal rats. The most significant ($p<0.05$) increases in the haematological profile was induced by oral treatment with 200 mg/kg HU (Table 1).

3.3. Effect of 28 days of oral HU treatment on the haematological parameters of cyclophosphamide-treated rats

Similar improvement in the haematological profile was observed on the 25th day of oral treatment with HU, before the commencement of treatment with cyclophosphamide which was given intraperitoneally. Daily treatment of rats with 30 mg/kg/day cyclophosphamide for 3 days caused a significant ($p<0.05$) decrease in the haematological parameters when compared to their values on the 25th day. However, oral pre-treatment with 50-200 mg/kg HU significantly ($p<0.05$) attenuated reductions in the values of the haematological parameters,

with the highest dose of the extract offering the most significant protection (Table 2).

4. Discussion

The present study investigates the effect of repeated oral treatment with 50-200 mg/kg/day HU on the haematological profile in normal and cyclophosphamide treated rats. Cyclopho-sphamide and other analogues of alkylating anticancer drugs (such as mechlorethamine, melphalan, cyclopho-sphamide, ifosphamide, chlorambucil, carmustine, busulphan, etc.) induce their cytotoxic actions by interfering with DNA synthesis and cell division [16, 17]. The cytotoxic actions of alkylating agents are reported to be non-selective particularly on rapidly proliferating tissues in which a large proportion of the cells are in active phase (G1/ S) of cell division [17, 18]. Body tissues with high mitotic indices include the bone marrow, gastrointestinal tissues and the gonads. In the present study, treatment of rats with a high dose cyclophosphamide resulted in pancytopaenia which is in agreement with that earlier reported by Manjrekar et al. [15]. Oral treatment with HU appears to have selective stimulatory or enhancing activity on the haemoglobin concentration, packed cell volume, platelet count, total leucocyte count and lymphocyte differentials in both normal and cyclophosphamide-treated rats, suggesting that HU could have selective stimulatory activity on the progenitors of these cell lines. However, the stimulatory effect of HU on the RBC Hb, and PCV is at variance with previous report where 14 days of oral treatment with 0.5 ml-1.0 ml of the water seed extract of *Hunteria umbellata* in New Zealand rabbits resulted in non-significant elevation in the RBC, Hb, PCV and TLC values [19]. The variation could have resulted from differences in dose and duration of treatment. Conversely, the relative lymphocytosis obtained for HU in this study is in accord with that previously reported by Ibeh et al. [19]. In view of the protective effect of HU on the haematological parameters of cyclophosphamide-treated rats, it is plausible that HU could be mediating its protective action via stimulation of DNA synthesis or DNA repairs.

HU has been previously reported to contain secondary metabolites such as alkaloids, flavonoids, saponin, cardiac glycosides, anthocyanosides, tannins and reducing sugars [12]. It has also been reported that plant extracts with notable haematopoietic activities are rich in phenolic, flavonoids and alkaloids which account for their haematopoiesis and immunomodulatory activities [15]. Thus, the presence of flavonoids, alkaloids and other phenolic compounds in HU could account for the observed stimulatory activity in some of the measured haematological parameters.

5. Conclusion

Results of this study showed that the body mechanism of HU-pretreated normal and cyclophosphamide-treated rats responded to the drug-induced pancytopaenia by stimulating and/ or preventing the cytotoxic effect of cyclophosphamide on the haematopoietic tissues. Thus, HU could become useful in the treatment of anaemia and in preventing drug-induced anaemia in patients on cancer chemotherapy.

6. Acknowledgements

The authors acknowledge with profound gratitude the technical assistance of Mr. M.O. Arogundade, Department of Haematology and Blood Transfusion, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria.

References

1. World Health Organization. (1991) *WHO* (EB 89/ 27), Geneva, Switzerland.

2. Agbor AG, Odetola AA. (2001) *African Journal of Medicine and Medical Sciences* 30: 105-109.

3. Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. (1997) *American Journal of Clinical Nutrition* 65: 153-159.

4. Awasthi S, Bundy D. (2007) *British Medical Journal* 334 (7603): 1065-1066.

5. Adeneye AA. (2008) *Biomedical Research* 19(1): 23-26.

6. Guralnik JM, Eistenstaedt RS, Ferruci L, Klein HG, Woodman RC. (2004) *Blood* 104: 2263-2268.

7. Falase AO, Akinkugbe OO. (1999) *A Compendium of Clinical Medicine*, Spectrum Books Limited: Ibadan, Nigeria; 545-604.

8. World Health Organization. (1996) *Technical Report Series* No. 863.

9. Nwabuisi C. (2002) *East African Medical Journal* 79(7): 343-346.

10. Adekoge EA, Alo B. (1986) *Phytochemistry* 25(6): 1461-1468.

11. Falodun A, Nworgu ZAM, Ikponmwonsa MO. (2006) *Pakistani Journal of Pharmaceutical Sciences* 19(3), 256-258.

12. Adeneye AA, Adeyemi OO. (2009a) *International Journal of Applied Research in Natural Products* 2(1): 9-18.

13. Adeneye AA, Adeyemi OO. (2009b) *Journal of Ethnopharmacology*, 126(2): 238-243.

14. United States National Institutes for Health publication. (1985) no. 85-23.

15. Manjrekar PN, Jolly CI, Narayanan S. (2000) *Fitoterapia* 71: 254-257.

16. Burger PM, Projan SJ, Horwitz SB, Peisach J. (1986) *Journal of Biological Chemistry* 261: 15955-15959.

17. Chabner BA, Ryan DP, Paz-Ares L, Garcia-Carbonero R, Calabresi P (2001). *In: Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill Medical Publishing Division: New York; 1389-1459.

18. Begleiter A, Glazer RI, Israels LG, Pugh L, Johnston JB. (1987) *Cancer Research* 47: 2498-2503.

19. Ibeh IN, Idu M, Ejimadu IM. (2007) *Bioci-ncias* 15(1): 4-7.