

1 ERYTHROPOIETIC AND BONE MARROW STIMULATING ACTIVITY OF
2 *TERMINALIA CATAPPA* EXTRACT: POSSIBLE ROLE OF NITRIC OXIDE
3 SIGNALING

4
5
6 ABSTRACT

7 *Terminalia catappa* is used commonly for the management of sickle cell anemia in
8 Nigeria. We assessed the capacity and mechanism of *Terminalia Catappa* (TC) to induce
9 erythropoiesis *in vivo* in phenyl hydrazine induced anemic mice. TC was able to up-
10 regulate the synthesis of intracellular hemoglobin 0.135 ± 0.004 significantly comparable to
11 hydroxyurea (HU) 0.158 ± 0.006 and normalize the peripheral blood reticulocyte index and
12 bone marrow carbonic anhydrase activity significantly at $P < 0.05$ 0.94 ± 0.25 close to the
13 non anemic mice 0.97 ± 0.25 . TC induced nitric oxide synthase activity significantly
14 ($P < 0.05$) comparable to hydroxyurea. The results also demonstrate that TC supports
15 normal erythroid differentiation *in vivo* in phenylhydrazine induced anemic mice in a
16 synergistic fashion. These suggest potential ethnopharmacological benefits which are
17 discussed herein.

21

22 Keywords: *Terminalia catappa*, Nitric oxide, arginase, phenylhydrazine, sickle cell
23 anemia, erythropoiesis and hemolytic anemia

24

25

26 INTRODUCTION

27 *Terminalia catappa* (almond) from the family combrataceae is known to originate from
28 sub-tropical zones of India and Indonesia. Almond is known to have a barrage of medicinal
29 uses; anti-inflammatory uses, hepatoprotective, antioxidant, antiparasitic and antimicrobial
30 among other uses [Chu et al., 2007; Kinoshita et al., 2007; Babayi et al., 2004; Fan et al.,
31 2004]. In Nigeria, the ethanol extracts of almond leaf have shown potential in treating
32 sickle cell anemia.

33 Anemia could result from rapid loss of red blood cells (RBC) which is a common feature
34 of SCA and the β -thalassemia or ineffective RBC synthesis from the bone marrow [Esbach
35 et al., 1987], usually a balance in synthesis and depletion of RBC is the key at maintaining
36 a healthy hemoglobin level. Nitric oxide (NO) serves as a potent signaling molecule
37 causing vasodilation and increased local blood flow [Kim et al., 2007; Etten et al., 2002;
38 Spieker et al., 2002]. It has been shown previously that its level is decreased in chronic
39 hemolytic anemia like sickle cell anemia (SCA) and β -thalassemia cases [Perrine, 2011;
40 Eberhardt et al., 2003; King, 2003; Morris et al., 2003]. In addition, NO has been

implicated in hydroxyurea (HU) and hydroxybutyrate induced gamma globin gene activation [Bianchi et al., 2007; King, 2003; Cokic et al., 2003].

Erythropoiesis involves the proliferation and differentiation of pluripotent stem cells into immature erythroid progenitors and finally into reticulocytes which usually takes place in the bone marrow in adult primates [Maria et al., 1999; Kenedy et al., 1997]. Studies into the mechanisms of proliferation of the erythroid cells has implicated NO as a major candidate inducer and high NO levels have been shown to be associated with heightened erythropoietic activity, [Hsu et al., 2006; Beleslin-cokic et al., 2004]. The availability of NO in the blood is modulated by the activity of arginase which depletes plasma arginine the substrate for NO production by NO synthase [Little et al., 2009; Kato et al., 2007; Chang et al., 1998].

Pharmacologic mediation of improved fetal hemoglobin synthesis through the induction of NO could be of interest in the chemotherapy of hemolytic anemia like sickle cell anemia and β -thalassemias [Fibach et al., 2003]. We have shown previously that TC methanolic extract enhances intracellular hemoglobin content in Balb C mice [Aimola et al., 2011], but the precise mechanism is not known. To investigate this we evaluated TC extract potency in stimulating bone marrow erythropoietic activity in phenyl hydrazine induced anemic Balb C mice.

62 MATERIALS AND METHODS

63 TC was obtained from Botanical garden of Ahmadu Bello University, Zaria (Longitude
64 7.72518 latitude 11.11324). It was identified at the Department of Biological Sciences
65 Herbarium by U.S. Gallah and deposited with voucher number 3940-TC.

66 **Fractionation of *Terminalia cattapa* L.**

67 The fresh leaves of TC were oven dried to a constant weight at 27°C in a Gallenkamp
68 OGS60 oven for 24hrs and the dried leaves were then pulverized, then 50g was extracted
69 exhaustively with 200ml methanol and distilled water and subsequently filtered using a
70 Whatman No.1 filter paper and the filtrate was concentrated at 40°C. The extract was
71 reconstituted in 10ml of methanol and loaded on a pre-equilibrated silica gel column. The
72 extract was then partitioned according to solubility in different solvents as previously
73 described [Akiyama et al., 2005; Newton et al., 2002]. The active fractions were eluted
74 using 20ml of analytical grade petroleum ether, methanol, ethanol, acetone (Pharmacia).
75 The eluate was air dried and stored in amber bottles.

76 **Animals**

77 This study was conducted according to international ethical principles and guidelines for
78 animal studies. Thirty six apparently healthy Balb C mice weighing 20-25g/mice were
79 housed in well ventilated cages in the Animal house of the Department of Pharmacology,
80 Ahmadu Bello University, Zaria and allowed to acclimatize for three weeks maintained on
81 conventional rat chow [Reaven et al., 2003] and water. The mice were divided into 3
82 control groups (A, B and C) and groups (D, E, F, G, H, I). Phenyl hydrazine (20mg/kg

bodyweight) was administered intraperitoneally to all the groups except group A. Group B was not treated for the duration of the experiment, group C was treated with hydroxyurea 15mg/kg bodyweight orally [Grant et al., 1997]. Groups D, E, F, G, H and I were treated with 43mg/kg bodyweight of the TC solvent fractions orally corresponding to one-fifth of the documented LD₅₀ of TC [Ahmed et al., 2005]. Group A received phosphate buffered saline only orally. The treatment regime was for 5 days and blood was collected in EDTA for further analysis. Bone marrow was also extracted from the femur of the mice as previously described [Zhang et al., 2007]. The PCV of the mice was assayed using standard procedures as previously described [Hanson et al., 1997].

Assay for Intracellular Hemoglobin and Reticulocytes

The number of reticulocytes in 10µl of blood from tail vein was determined using the methylene blue assay [Riley et al., 2001]. Intracellular hemoglobin was assayed by lysing RBCs using repeated freezing and thawing in distilled water and the lysate subjected to benzidine assay in 10mM phosphate buffer using hemoglobin as standard [Riley et al., 2001].

Arginase Assay

The arginase assay was carried out spectrophotometrically by measuring the formation of L-ornithine at 515 nm with ninhydrin as previously described [Hafid-Medheb et al., 2003] with slight modifications. 50µl serum samples were diluted to 1ml with 0.01mM carbonate bicarbonate buffer containing 0.13% v/v sodium azide and 0.43% w/v L-arginine. The

mixtures were incubated at 37°C for 20min. The reaction was stopped by cooling on ice and the addition of 2ml 0.71% ninhydrin containing 91% acetic acid v/v and 1.1% v/v phosphoric acid. Color formation proceeded for 15min at 95°C. The concentration of L-ornithine was measured spectrophotometrically at 515 nm in 1.5 ml cuvettes. Representative data are presented as activity in μmol L-ornithine/min

Carbonic Anhydrase Assay

Bone marrow Carbonic anhydrase (CA) was assayed using CO_2 saturated water as substrate as previously described [29]. Briefly 100 μl bone marrow extracted from the femur of the mice was re-constituted in Tris-HCl buffer pH 8.3 and maintained on ice, then 4ml of the substrate was then introduced, the reaction was monitored and time recorded for pH drop to 6.3. A similar setup (excluding the bone marrow) was carried out for the blank. The activity was expressed as units/ml of enzyme.

Results

***Terminalia catappa* supports normal erythroid differentiation in phenyl hydrazine induced anemic mice**

We assessed the ability of the different fractions of TC to stimulate normal erythroid differentiation by determining its effect on bone marrow erythroid cell carbonic anhydrase activity. Bone marrow carbonic anhydrase activity was significantly suppressed ($p < 0.05$)

in groups administered with methanolic TC methanolic fraction (1.503 ± 0.09 unit/ml), methanolic TC petroleum ether fraction (1.218 ± 0.1 unit/ml), methanolic TC acetone fraction (0.666 ± 0.005 unit/ml), Aqueous TC Ethanolic fraction (1.019 ± 0.209 unit/ml) aqueous TC petroleum ether fraction (0.966 ± 0.02 unit/ml) and aqueous TC methanolic fraction (1.503 ± 0.123 unit/ml) when compared to the negative control (1.974 ± 0.06 unit/ml) as shown in Figure 3. While no significant difference was observed when compared to the normal control (1.075 ± 0.08 unit/ml) also there was no significant difference in group treated with hydroxyurea (1.589 ± 0.005 unit/ml) when compared to aqueous TC methanolic fraction (1.503 ± 0.09 unit/ml). TC appeared to normalize bone marrow carbonic anhydrase activity comparable to hydroxyurea treated mice.

***Terminalia catappa* Improves bone marrow activity in Balb C mice**

We assayed the ability of TC to restore normal bone marrow function in phenyl hydrazine induced anemic mice by assaying for its effect on number of circulating reticulocytes and total formed elements in the blood. We observed that TC equalized the number of circulating erythrocytes significantly comparable to that of the normal (none anemic) mice and hydroxyurea treated mice. The proportion of reticulocytes in the peripheral circulation was significantly elevated in the phenyl hydrazine induced anemic mice compared to the treated groups as shown in Figure 2. The PCV of the anemic mice treated with the different fractions of TC was significantly elevated above the untreated phenyl hydrazine induced anemic mice as shown in Figure 5.

145

146 ***Terminalia catappa* improves hemoglobin synthesis**

147 TC increased the content of intracellular hemoglobin significantly higher 0.135 ± 0.001
148 than the phenyl hydrazine induced anemic untreated mice 0.00995 ± 0.003 and the normal
149 untreated control mice 0.078 ± 0.002 as shown in Figure 1.

150 ***Terminalia catappa* increases production of Nitric Oxide *in vivo***

151 NO has been implicated in increased fetal hemoglobin synthesis. NO production has been
152 shown to be modulated by the activity of arginase. We studied the ability of TC to function
153 as a stimulant for production of NO by assaying for its effect on serum arginase activity.
154 The results revealed a serum arginase suppressive activity of the solvent fractions of TC,
155 comparable to the control erythropoietic drug; hydroxyurea as shown in Figure 4.

156

157 **Discussion**

158 Hemolytic anemia is a condition in which there are not enough RBCs in the blood due to
159 premature destruction of the RBCs or blood loss from the body. Anemia ensues when the
160 bone marrow cannot effectively make up for the lost RBCs, similarly as is found in sickle
161 cell hemolytic anemia there exists an insufficiency in the synthesis of RBC from the bone
162 marrow [Mcintosh, 1968]. Herein we demonstrate for the first time the capacity for active
163 fractions of TC to induce erythropoiesis in bone marrow through a NO signaling pathway.

We found out that the active fractions of *Terminalia catappa* increased intracellular hemoglobin significantly above the untreated mice and non hemolytic mice but quite similar to the pattern of increase found in mice treated with HU this is consistent with our previous finding that the crude extract of TC induces hemoglobin synthesis [Aimola et al., 2011].

Active hemoglobin synthesis occurs in committed erythroid cells, and is accompanied with increased synthesis of carbonic anhydrase in normal erythroid development. Consequently carbonic anhydrase expression is linked to the fetal hemoglobin (HbF) to adult hemoglobin (HbA) switch at the late phase of erythroid development [Hebbel and Miller, 1984]. We observed the active fractions appeared to lower the expression of carbonic anhydrase significantly comparable to the normal non anemic non treated mice as measured by the appearance of CA activity although this was not the case when phenyl hydrazine induced hemolytic anemic mice were treated with HU a fetal hemoglobin inducer which increased the expression of CA significantly above the phenyl hydrazine induced hemolytic untreated mice. This demonstrates that HU and the plant extracts induce normal erythroid development in Balb C mice through different mechanism which requires further studies.

To further understand if the active plant fractions of TC can support reversal of the HbF to HbA switch, and possibly with a potential to induce fetal hemoglobin synthesis in SCD patients who commonly use this plant as a local treatment in the northern region of Nigeria, we tested its ability to induce the production of NO by assaying its activity on blood arginase activity. NO has been demonstrated previously to be involved in the activation of λ -globin activation through the activation of soluble guanyl cyclase [Cokic et

al., 2003]. In addition Nitric oxide synthase activity has been shown to be modulated by arginase which competes with the enzyme for L-arginine the physiologic precursor of NO [Laminkra et al., 2009; Durante et al., 2007; Chang et al., 1998; Hey et al., 1997; Brady et al., 1990]. The study revealed that active fractions of TC suppressed serum arginase activity significantly higher than HU which is indicative of a potential of TC as a possible potentiating agent for HbF synthesis [Cokic et al., 2003].

We analysed for the possibility of an in vivo dyserythropietic response to TC by the mice in comparism to HU which have been shown to stimulate normal erythropoietic response in bone marrow of mice [Hey et al., 1997]. A significantly improved reticulocyte response was observed with administration of TC similar to HU as shown in Figure 3

HU stimulates the synthesis of fetal hemoglobin through an induced bone marrow erythroid development with an increased erythropoietic response and has been demonstrated to be therapeutic and highly useful in hemolytic blood disorders like sickle cell anemia and β -thalassemia [Cokic et al., 2003; Hey et al., 1997]. The improved erythropoietic response in mice with hemolytic anemia due to TC that we describe here underscores a huge potential of TC active fractions activity in hemolytic blood disorders. TC appeared to suppress the expression of CA in the later phase of erythroid development suggesting the presence of components that masks the expression of CA in TC. The experimental model described herein revealed little or no side effects associated with administration of TC leaf extracts. Further trials are required though to establish a dose dependent response to TC and to establish the structure and chemical nature of the components of the active fractions of TC.

Acknowledgement; The authors would like to acknowledge the Chief Technologist Mr. Stephen Jock and his co-technologists for the immense assistance provided in some of the logistics required for the completion of this study.

Reference

- Ahmed S.M., Bm V.S., Dhanapal P.G.R., Chandrashekara V.M. (2005). *Ira. J. Pharm. and Ther.* 4; 36-39.
- Aimola I.A., Inuwa H.M., Mamman A.I., Habila N., Agbaji A.S., Omoniwa D. (2011). *Terminalia catappa* Extract Enhances Erythropoiesis in Adult *Balb C* mice. *J. Mol. Biol. Res.* Vol. 1 (1) 40-46. doi:10.5539/jmbr.v1n1p40
- Akiyama K., Matsuzaki K., Hayashi H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435, 824-827. doi:10.1038/nature03608.
- Babayi H., Kolo I., Okogun J. I. & Ijah U. J. J. (2004). The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri*, 16(2),106-111.
- Beleslin-cokic B.B., Cokic V.P., Yu X., Weksler B.B., Schecter A.N. and Noguchi C.T. (2004). Erythropoietin and hypoxia stimulate nitric oxide production by endothelial cells. *Blood*. 104(7); 2073-2080. doi: 10.1182/blood-2004-02-0744
- Bianchi N., Zuccato C., Lampronti I., Borgatti M. and Gambari R. (2007). Fetal globin inducers from the natural world: A novel approach for the identification of drugs for the treatment of β -thalassemia and sickle cell anemia. *eCAM*. doi:10.1093/ecam/nem139

- Brady H.J.M., Edwards M., Linch D.C., Knott L., Barlo J.H., Butterworth P.H.W. (1990). Expression of the human carbonic anhydrase I gene is activated late in fetal erythroid development and regulated by stage specific trans acting factors. *Br.J. of Haematol.* 76(1). 135-142.
- Chang CI, Liao JC, Kuo L. (1998). Arginase modulates nitric oxide production in activated macrophages. *Am. J. of Physiol. Heart Circ. Physiol.*;274:H342–H348.
- Chu S. C, Yang S. F, Liu S. J, Kuo W. H, Chang Y. Z & Hsieh Y. S. (2007). In vitro and in vivo antimetastatic effects of *Terminalia catappa* L. leaves on lung cancer cells. *Food and chem. Toxicol.*, 45(7), 1194-1201. <http://dx.doi.org/10.1016/j.fct.2006.12.028>
- Cokic V.P., Smith R.D., Beleslin-Cokic B.B., Njoroge J.M., Miller J.L., Gladwin M.T. and Schechter A.N. (2003) Hydroxyurea induces fetal hemoglobin by the nitric oxide–dependent activation of soluble guanylyl cyclase. *J. Clin. Investig.* 111:231–239. doi:10.1172/JCI200316672.
- Durante W., Johnson F.K. and Johnson R.A. (2007) Arginase: A Critical Regulator of Nitric oxide synthesis and vascular function. *Clin. Exp. Pharm.and Physiol.*. 34(9): 906-11.
- Eberhardt R.T., McMahon L., Duffy S.J., Steinberg M.H., Perrine S.P., Loscalzo J., Cofman J.D. and Vita J.A. (2003). Sick cell anemia is associated with reduced Nitric oxide bioactivity in peripheral conduits and resistance vessels. *Am. J.of Heamatol.* 74:104–111
- Eschbach M.D., Joan C.E., Downing M.R., Browne J.K., Adamson J.W., (1987). Correction of the Anemia of End-Stage Renal Disease with Recombinant Human Erythropoietin. *New Eng.J.Med.* 316:73-7 .
- Etten R.V., deKoning E., Verhaar M., Gaillard C. and Rabelink T. (2002) Impaired NO-dependent vasodilation in patients with Type II (non-insulin-dependent) diabetes mellitus is restored by acute administration of folate. 2002. *Diabetologia* . Volume 45, Number 7, 1004-1010, DOI: 10.1007/s00125-002-0862-1.
- Fan, Y. M., Xu, L. Z., Gao, J., *et al.* (2004). Phytochemical and anti-inflammatory studies on *Terminalia catappa*. *Fitoterapia*, (75), 253-260. <http://dx.doi.org/10.1016/j.fitote.2003.11.007>
- Fibach E., Bianchi N., Borgatti M., Prus E. and Gambari R. (2003) Mithramycin induces fetal hemoglobin production in normal and thalassemic human erythroid precursor cells. *Blood* ;102:1276-1281

- Grant C.A., Ponnazhagen S., Wang X., Srivastava A. and Li T. (1997). Evaluation of recombinant adeno-associated virus as a gene transfer vector for the retina. *Curr.Eye Res.* 16. (9). 949-956. doi/abs/10.1076/ceyr.16.9.949.5046.
- Hafid-Medheb K., Bourget Y.A., Minatchy M.N., Hanania N. and Lezene J.R. (2003). Bcl-XL is required for heme synthesis during the chemical induction of erythroid differentiation of murine erythroleukemia cells independently of its antiapoptotic function. *Blood.* Volume 101 (7). 2575-2583
- Hanson S.M., Botswick D.R., Twedt D.C. Smith M.O. (1997) Clinical evaluation of cimetidine, sucralfate, and misoprostol for prevention of gastrointestinal tract bleeding in dogs undergoing spinal surgery. *Am. J. Vet. Res.* 58 (11) 1320-3.
- Hebbel R.P. and Miller W.J. (1984). Phagocytosis of sickle cell erythrocytes; immunologic and oxidative determinants of hemolytic anemia. *Blood.* 64. 733-741
- Hey C, Boucher JL, Vadon-Le Goff S, Ketterer G, Wessler I, Racke K. Inhibition of arginase in rat and rabbit alveolar macrophages by N -hydroxy-D,L-indospicine, effects of L-arginine utilization by nitric oxide synthase. *Br J Pharmacol* 1997;121:395–400.
- Hsu L.L., Champion H.C., Campbell-Lee S.A., Bivalacqua T.J., Manchi E.A., Diwan B.A., Schimel D.M., Cochard A.E., Wang S., Schechter A.N., Noguchi C.T. and Gladwin M.T. (2006). *Blood.* 109(7); 3088-3098. doi: 10.1182/blood-2006-08-039438
- Kato G.J., Gladwin M.T. and Steinberg M.H. (2007). Deconstructing sickle cell disease: Reappraisal of the role of hemolysis in the development of clinical sub-phenotypes. doi: 10.1016/j.blre.2006.07.001
- Kennedy M., Firpo M., Choi K., Wall C., Robertson S., Kabrun N. and Keller G. (1997). A common precursor for primitive Erythropoiesis and definitive hematopoiesis. *Nature.* 386, 488-496.
- Kim J., Formoso G., Li Y., Potenza M.A., Marasciulo F.L., Montagnani M. and Quon M.J., (2007) Epigallocatechin Gallate, a Green Tea Polyphenol, Mediates NO-dependent Vasodilation Using Signaling Pathways in Vascular Endothelium Requiring Reactive Oxygen Species and Fyn. *J. Biol. Chem.* 282, **13736-13745.** doi: 10.1074/jbc.M609725200.
- King S. B. (2003) A role for nitric oxide in hydroxyurea-mediated fetal hemoglobin induction. *J. of Clin. Investig.* 111:171–172. doi:10.1172/JCI200317597.
- Kinoshita S, Inoue Y, Nakama S, Ichiba T & Aniya Y. (2007). Antioxidant and hepatoprotective actions of medicinal herb, *Terminalia catappa L* from Okinawa Island

and its tannin corilagin. *Phytomedicine*, 14, 755-62.

<http://dx.doi.org/10.1016/j.phymed.2006.12.012>

Laminkra AA, Theron M, Kooij TWA, Roberts DJ (2009) Hemozoin (Malarial Pigment) Directly Promotes Apoptosis of Erythroid Precursors. *PLoS ONE* 4(12): e8446. doi:10.1371/journal.pone.0008446.

Little J.A., Hauser K.P., Matyr S.E., Harris A., Maric I., Morris C.R., Suh J.H., Taylor J., Castro O., Machado R., Kato G., Gladwin M.T. (2009). Hematologic, biochemical and cardiopulmonary effects of L-arginine supplementation or phosphodiesterase-5 inhibition in patients with sickle cell disease who are on hydroxyurea therapy. *Eur.J.Haematol.* 82(4). 315-321. doi:10.1111/j.1600-0609.2009.01210.x.

Maria D.R., Zeuner A., Eramo A., Domenichelli C., Bonchi D., Grignani F., Srinivasula S.M., Alnemri E.S., Testa U., Peschle C. (1999). Negative regulation of Erythropoiesis by caspase mediated cleavage of GATA-1. *Nature* **401**, 489-493.

McIntosh J.E.A.(1968). Assay of Carbonic Anhydrase by Titration at Constant pH. *Biochem.J.* (109), 203.

Morris C.R., Morris S.M., Hagar W., Warmedam J.V., Claster S., Lenhardt D.K., Machado L., Kuypers F. A. and Vichinsky E. P. (2003). Arginine therapy; A new treatment for pulmonary hypertension in sickle cell disease. *Am. J. of Res. and Crit. Care Med.* 168. pp 63–69. DOI: 10.1164/rccm.200208-967OC

Newton S.M., Lau C., Gurucha S.S., Besra G.S., Wright C.W. (2002). The evaluation of forty-three plant species for in vitro antimycobacterial activities; isolation of active constituents from *Psoralea*

Perrine S. (2011). Switching globins; raising red cells. *Blood*. 118: 834-836. doi:10.1182/blood-2011-06-354373

Reaven E., Wright D., Mondon C.E., Solomon R., Ho H. and Reaven G.M. (1983). Effect of age and diet on insulin secretion and insulin action in the rat. *Diabetes*. Vol 32 (2). 175-180

Riley R.S., Ben-Ezra J.M., Tidwell A. (2001) Reticulocyte Enumeration: Past & Present. *Laboratorymedicine*. volume 32 (10) 599-608

Spieker L.E., Isabella S., Hurlimann D., Lerch P.G., Lang M.G., Bingeli C., Corti R., Ruschitzka F., Luscher T.F., and Noll G. (2002) High-Density Lipoprotein Restores Endothelial Function in Hypercholesterolemic Men. *Circulation*. **105**: **1399-1402** doi: 10.1161/01.CIR.0000013424.28206.8F

Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336:1–17.

Zhang J, Zhang X, Wu C, Lu D, Guo G, et al. (2011) Expression, Purification and Characterization of Arginase from *Helicobacter pylori* in Its Apo Form. *PLoS ONE* 6(10): e26205. doi:10.1371/journal.pone.0026205

Zhang X., Ma Y. and Zhang J. (2007) Human erythroid progenitors from adult bone marrow and cord blood in optimized liquid culture systems respectively maintained adult and neonatal characteristics of globin gene expression. *Bio. Res.* 40: 41-53.

UNDER PEER REVIEW