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In vitro Evaluation of Hydroxyl and Nitric Oxide Radical Scavenging Activities of Artemether

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Abstract: The hydroxyl and nitric oxide radical scavenging activities and total phenolic content of artemether were investigated *in vitro*. The concentrations of artemether (200-1000 μg mL^{-1}) did not exhibit hydroxyl radical scavenging activity except at 200 μg mL^{-1} concentration. The mean values for the hydroxyl radical scavenging for the different concentration (200, 400, 600, 800 and 1000 μg mL^{-1}) of artemether were 47.55±11.28, -3.49±13.16, -30.09±6.09, -34.74±4.29 and -49.00±7.75%, respectively. The total phenolic content of artemether in ethylacetate ranged from 208.00±48.33 (SD) to 340.00±14.14 μg mL^{-1}, while the total phenolic content of artemether in methanol ranged from 90.00±6.3 (SD) to 122.00±35.44 μg mL^{-1}. Total phenolic content of artemether was significantly higher in ethyl acetate than in methanol at all concentration investigated ($p<0.05$). The total phenolic content arthemeter was influenced by the organic solvent used as a medium for preparing artemether.

Key words: Free radical, pathophysiology, Artemisinins, *in vitro*, reactive oxygen and nitrosative stress, Nigeria

INTRODUCTION

Artemisinin or Qinghaosu is an extract of medicinal plant Qinhao (Artemisia annua) (Novartis, 2005), a herb which has been used in traditional Chinese medicine for over 2000 years (Winstanley *et al*., 2004). Artemisinin is a sesquiterpene lactone with a peroxide bridge and its antimalarial activity has been attributed to its peroxide moiety (Klayman, 1985) that generates free radicals which damages the parasite membrane (Meshnick *et al*., 1989). Artemisinin compounds include artemether, artesunate and dihydroartemisinin (Mueller *et al*., 2004). Artemisinin has no nitrogenous heterocyle (Schmid and Hofheinz, 1983).

Artemether is an oil-soluble derivative of artemisinin of sesquiterpene lactone peroxide linkages (White and Olliaro, 1998; Hien and White, 1993). Its chemical structure is decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano-1, 2-benzodioxepin and pharmacological name is artemetherum (Utzinger *et al*., 2001). Artemisnins which do not possess peoxidic oxygen are devoid of antimalarial activity (Avery *et al*., 1992). Amorpha -4, 11-diene synthase is a key enzyme for artemisinin synthesis (Bouwmeester *et al*., 1999). Artemisinins possess antimalarial (Woodrow *et al*., 2005), antitumor (Efferth *et al*., 2002), antibacterial, antiviral (Efferth *et al*., 2007), immunosuppressive (Zhang *et al*., 2002; Tam *et al*., 2000) activities. Hitherto, there is no scientific study on *in vitro* hydroxyl and nitric oxide radical scavenging activities of artemether. This study was therefore carried out to assess the *in vitro* hydroxyl and nitric oxide radical scavenging activities of artemether and assess the relationships between hydroxyl radical scavenging ability, nitric oxide scavenging activity and total phenolic concentration of artemether.

MATERIALS AND METHODS

Chemical reagents: Folin-Ciocalteau reagent, $FeSO_4.7H_2O$ and 1,10-Phenanthroline used were products of British Drug House (BDH), UK.

Sample: The brand of Artemether used was Lariether brand with a stock concentration of 40 mg mL^{-1}. The drug was purchased from Taki Medicine Store, Ogbomoso, Oyo State, Nigeria.

Preparation of the different concentrations of artemether from the stock conc. of 40 mg mL^{-1}: About 200-1000 ug mL^{-1} concentrations of artemether were prepared in ethylacetate and methanol from the stock concentration of artemether (40 mg mL^{-1}).

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Biochemical assays

Total phenolics content estimation: Total phenolic content of artemether was estimated according to the method of Hung *et al.* (2002). This is based on the principle that the phenolic group present in the sample reacts with Folin-Ciocalteau reagent in an alkaline medium using Sodium Carbonate (Na_2CO_3) solution thus giving a blue colour. About 0.5 mL of Folin-Ciocalteau (10%, w/v aqueous) was added to 0.1 mL of each concentrations of artemether, followed by the addition of 0.4 mL of aqueous Na_2CO_3 (7.5% w/v). The mixture was allowed to stand in the dark for 30 min. The absorbance of the blue color solution was read at 765 nm on a UV visible spectrophotometer (Genesy 10 vis, Thermoelectric corporation, USA) against blank (distilled water). Total phenolic concentration (mg mL^{-1}) of the artemether was extrapolated from a standard curve using tannic acid as a standard.

Nitric oxide scavenging activity: The nitric oxide radical scavenging activity of artemether was assayed according to the method of Marcocci *et al.* (1994). Nitric oxide was generated from sodium nitroprusside and the nitrite formed was measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Green *et al.*, 1982; Marcocci *et al.*, 1994) which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Ruch *et al.*, 1989). About 0.1 mL aqueos sodium nitroprusside and 0.2 mL of 0.2M Na_2HPO_4 (pH 7.8) were added to 0.4 mL of different concentrations of artemether and the mixture incubated at room temperature for 2 h and 30 min. About 0.12 Griess reagent was added and the absorbance of the pink colour was read at 540 nm against blank (distilled water).

$$\text{Nitric oxide redical scavenging (\%)} = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

Where:

$A_{control}$ = Absorbance of the control in the absence of sample

A_{sample} = Absorbance of sample

Hydroxyl radical scavenging activity assay: The scavenging capacity for hydroxyl radical of artemether was assayed according to the method of Yu *et al.* (2004). Hydroxyl radical is generated *in vitro* by mixing iron (II) sulphate which generates ferrous ion (Fe^{2+}) with hydrogen peroxide and 1, 10-phenanthroline. The 1, 10-phenanthroline was used since phenanthroline-Fe^{2+} is a commonly used indicator of redox reaction. The H_2O_2/Fe^{2+} system produces hydroxyl radical through the Fenton reaction and phenanthroline-Fe^{2+} complex oxidizes to Fe^{3+}. The hydroxyl radical produced can then be determined due to a change in absorbance at 560 nm. About 60 uL of $FeSO4.7H_2O$ (1 mM) was added to 90 uL of aqueous 1, 10-phenanthroline (1 mM). About 2.4 mL of 0.2M Na_2HPO_4 (pH 7.8) was added to the mixture, followed by the addition of 150 uL of H_2O_2 (0.17 M) and 1.5 mL of different concentrations of artemether (250-1000) in sequence. The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm after 5 mm on the UV/visible spectrophotometer (Genesy 10 vis, Thermoelectric corporation, USA) against blank (distilled water).

$$\text{Hydroxyl redical scavenging (\%)} = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

Where:

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the sample in the presence of other reagents in the reaction mixture

RESULTS AND DISCUSSION

Artemether was observed to exhibit moderate *in vitro* hydroxyl radical scavenging activity (47.55%) at the lowest concentrations of arthemeter (200 μg mL^{-1}). At other concentrations (400-1000 μg mL^{-1}) of artemether, the drug failed to scavenge hydroxyl radical *in vitro*. The drug at those concentrations (400-1000 μg mL^{-1}) accelerated hydroxyl production as evidenced by their negative values. At all concentrations investigated (200-1000 μg mL^{-1}), artemether did not exhibit nitric oxide radical activity *in vitro* as evidenced by their negative values (Table 1). The drug accelerated the generation of nitric oxide radical *in vitro*. The solvent used in preparing artemether before total phenolic determination influenced the total phenolic content of artemether. The total phenolic content artemether in ethyl acetate was significantly higher than the total phenolic content ($p<0.05$) in methanol at all concentrations investigated *in vitro* (200-1000 μg mL^{-1}). The total phenolic content of artemther in ethylacetate ranged from 208.00±48.33 to 340.00±14.14 (SD) mg mL^{-1}, while the total phenolic content of artemether in methanol ranged from 90.00±6.33 to 122.00±35.44 (SD) mg mL^{-1}. Hydroxyl radical scavenging activity of artemether in ethyl acetate showed a strong non-significant negative correlation with total phenolics concentration of artemether ($r = -0.972$; $p = 0.05$) at 200 μg mL^{-1} concentration (Table 2).

Table 1: Changes in levels of hydroxyl and nitric oxide radical scavenging activities and total phenolic concentration of artemether *in vitro* (in ethylacetate and methanol)

Concentration ($\mu g\ mL^{-1}$)	Hydroxyl radical scavenging ability (%)	Nitric oxide scavenging ability(%)	Total phenolic content (in ethyl acetate solvent) ($mg\ mL^{-1}$))	Total phenolic content (methanolic solvent)($mg\ mL^{-1}$))
200	47.55±11.28	-16.17±19.04	208.00±48.33	104.00±38.78
400	-3.49±13.16	-17.48±37.69	272.00±48.33	90.00±6.3300
600	-30.09±6.090	-39.59±13.88	328.00±27.13	122.00±35.44
800	-34.74±4.290	-38.02±25.17	340.00±14.14	92.00±26.380
1000	-49.00±7.750	-39.82±32.22	293.00±57.76	120.00±43.82

Values are mean±S.D of 5 analyses per concentration

Table 2: Pearson correlation of hydroxyl radical scavenging ability and total phenolics of artemether in ethyl acetate

Concentration of artemether ($\mu g\ mL^{-1}$)	Correlation (r)	t-value	p = 0.1	p = 0.05	p = 0.01	p = 0.001
200	-0.382	-7.687	NS	NS	NS	NS
400	-0.119	-8.946	NS	NS	NS	NS
600	0.272	-27.322	NS	NS	NS	NS
800	0.637	-38.777	NS	NS	NS	NS
1000	-0.444	-8.573	NS	NS	NS	NS

Table 3: Pearson correlation of total phenolic content in ethyl acetate and nitric oxide radical scavenging activity of artemether

Graded doses of artemether ($\mu g\ mL^{-1}$)	Correlation (r)	t-value (t)	p = 0.1	p = 0.05	p = 0.01	p =0.001
200	-0.972	-5.407	NS	NS	NS	NS
400	0.032	-11.090	NS	NS	NS	NS
600	0.932	-33.204	NS	NS	NS	NS
800	-0.090	-49.844	NS	NS	NS	NS
1000	0.644	-12.879	NS	NS	NS	NS

At 600 $\mu g\ mL^{-1}$ of artemether, a strong positive non-significant was observed between hydroxyl radical scavenging activity and total phenolics concentration of artemether in ethylacetate (r = 0.932; p = 0.05).

Nitric oxide radical activity of artemether in ethylacetate showed weak non-significant negative correlations with total phenolics at 200, 400 and 1000 $\mu g\ mL^{-1}$ of artemether (r = -0.382; p = 0.05 at 200 μg mL; r = -0.119; p = 0.05 at 400 $\mu g\ mL^{-1}$). A high positive non-significant correlation was observed between nitric oxide radical scavenging activity and total phenolics of artemether (r = 0.637; p = 0.05) (Table 3).

Hydroxy radicals can be generated from H_2O_2 in the presence of transition metals such as Fe^{2+} by Fenton reaction (Halliwell and Gutteridge, 1992). The short-lived hydroxyl radicals are assumed to be more unspecially destructive to biomolecules because they are located less than a few nanometers from the site of generation (Hipeli and Elstner, 1997).

Hydroxyl radical is generated *in vitro* by mixing iron (II) sulphate which generate ferrous ion (Fe^{2+}) with hydrogen peroxide and 1-10-phenanthroline (Liu, 2003). The 1,10-phenanthroline was used since phenanthroline-Fe^{2+} is a commonly used indicator of redox reaction. The H_2O_2/Fe^{2+} system produces hydroxyl radical through the Fenton reation with phenanthroline-Fe^{2+} complex oxidies to Fe^{3+} (Liu, 2003).

CONCLUSION

Artemether failed to scavenge nitric oxide radical at all the concentrations investigated *in vitro*. These findings suggested that artemether could be a potentiator of inflammation and it corroborated its traditional role as a free radical generator.

REFERENCES

Avery, M.A., W.K.M. Chong and C. Jenning-White, 1992. Sterioselective total synthesis of (+) -artemisinin, the antimalarial constituent of *Artemisia annua* L. J. Am. Chem. Soc., 114: 974-979.

Bouwmeester, H.J., T.E. Wallaart and M.H.A. Janssen, 1999. Amopha-4,11-diene synthase catalyses the first probable step in artemisinin biosynthesis. Phytochemistry, 52: 843-854.

Efferth, T., M. Davey, A. Olbrich, G. Rucker, E. Gebhart and R. Davey, 2002. Activity of drugs from traditional Chinese medicine toward sensitive and MDR1-or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukemia cells. Blood Cells Mol. Dis., 28: 160-168.

Efferth, T., M. Giaisi, A. Merling, P.H. Krammer and M. Li-Weber, 2007. Artesunate induces ROS-mediated apoptosis in doxorubicin-resistant T leukemia cells. PLoS ONE, 2: 693-693.

Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.K. Wishnok and S.R. Tannenbaum, 1982. Analysis of nitrate, nitrite and 15N in biological fluids. Anal. Biochem., 126: 131-136.

Halliwell, B. and J.M.C. Gutteridge, 1992. Biologically relevant metal ion dependent hydroxyl radical generation. An update. FEBS Lett., 307: 108-112.

Hien, T.T. and N.J. White, 1993. Qinghaosu. Lancet, 341: 603-608.

Hipeli, S. and E.F. Elstner, 1997. OH-radical-type reactive oxygen species: A short review on the mechanisms of OH-radical- and peroxynitrite toxicity. Z. Naturforsch C, 52: 555-563.

Hung, Y., V.M. Sava, S.Y. Makan, T.J. Chen, M. Hong and G.S. Huang, 2002. Antioxidant activity of melanins derived from tea, comparison between different oxidative states. Food Chem., 28: 233-240.

Klayman, D.L., 1985. Qinghaosu (artemismin): An antimalarial drug from China. Science, 228: 1049-1055.

Liu, J., 2003. The Pharmacological Experiment Methods New Technology Methods. Chemical Industry Press, China, pp: 97-98.

Marcocci, L., J.J. Maguire, M.T. Droy-Lefaix and L. Packer, 1994. The nitric oxide scavenging property of *Ginko biloba* extract EGB 761. Biochem. Biophys. Res. Commun., 201: 748-755.

Meshnick, S.R., T.W. Tsang, F.B. Lin, H.Z. Pan, C.N. Chang, F. Kuypers, D. Chiu and B. Lubin, 1989. Activated oxygen mediates the antimalarial activity of qinghaosu. Prog. Clin. Biol. Res., 313: 95-104.

Mueller, M.S., N. Runyambo and I. Wagner, 2004. Randomized controlled trial of a traditional preparation of *Artemisia annua* L. (Annual Wormwood) in the treatment of malaria. Trans. R. Soc. Trop. Med. Hyg., 98: 318-321.

Novartis, A., 2005. Coartem/riamet, Product monograph: A novel antimalarial combination: One product, two concepts. Novartis Pharm., 22: 123-129.

Ruch, R.J., S.J. Cheng and J.E. Klavning, 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens, 10: 1003-1008.

Schmid, G. and W. Hofheinz, 1983. Total synthesis of qinghaosu. J. Am. Chem. Soc., 105: 624-625.

Tam, L.S., D.D. Gladman, D.C. Hallett, P. Rahman and M.B. Urowitz, 2000. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. J. Rheumatol., 27: 2142-2145.

Utzinger, J., S.H. Xiao, J. Keiser, M.G. Chen, J. Zheng and M. Tanner, 2001. Current progress in the development and use of artemether for chemoprophylaxis of major human schistosome parasites. Curr. Med. Chem., 8: 1841-1860.

White, N.J. and P.L. Olliaro, 1998. Artemisinin and derivatives in the treatment of uncomplicated malaria. Med. Trop., 58: 54-56.

Winstanley, P., S. Ward, R. Snow and A. Breckenridge, 2004. Therapy of falciparum malaria in sub-Saharan Africa: From molecule to policy. Clin. Microbiol. Rev., 17: 612-637.

Woodrow, C.J., R.K. Haynes and S. Krishna, 2005. Artemisinins. Postgrad. Med. J., 81: 71-78.

Yu, W., Y. Zhao and B. Shu, 2004. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. Food Chem., 86: 525-529.

Zhang, J., J. Zhong, Z. Shi and X. Dai, 2002. Effects of lingdan and artesunate on T cell populations in human SLE. Zhongguo Zhong Xi Yi Jie He Za Zhi, 7: 489-491.