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Biochemical parameters as indicators of antihypertensive efficacy of stem bark extract of *Nauclea latifolia*

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ABSTRACT

Hypertension is a relatively common disorder that is probably the most important public health problem in developed countries. It is common, asymptomatic, readily detectable, usually easily managed and usually leads to lethal complications if left unmanaged. This study evaluates the serum electrolytes, lipid profile and enzymes activities as indicators of antihypertensive efficacy of stem bark extract of Nauclea latifolia. The extract produced a significant decrease ($p < 0.05$) of sodium in the treated animals compared to that of the hypertensive. The chloride levels in the treated and control groups were not significant ($p > 0.05$), and the potassium levels were insignificant in the treated and control groups ($p > 0.05$). There was a significant decrease ($p < 0.05$) in the Total Cholesterol levels in the treated groups compared to that of the hypertensive control, but were insignificantly higher ($p > 00.05$) than the normal control. The decrease changes in the Triacylglycerol levels of the treated groups were not significant ($p > 0.05$) when compared to the hypertensive control. The high density lipoprotein (HDL) levels in the treated groups and normal control were significantly increased ($p < 0.05$) compared to the hypertensive control. Also, the low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were similarly decreased in the treated groups, but these decreases were not significant ($p > 0.05$). The serum enzymes; Aspartate amino transferase and Alanine amino transferase though were increased, these increases were not significant ($p > 0.05$). These changes in the lipid profile, especially total cholesterol and high density lipoprotein show that the extract has antihypertensive properties. Also the reduced levels of these electrolytes, especially sodium showed antihypertensive efficacy of the extract.

Key words: *Nauclea latifolia*, antihypertensive indicators, biochemical paramaeters

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INTRODUCTION

Medicinal plants have served as valuable starting materials for drug development in both developing and developed countries [Wong et al, 2005]. Today, more than 80 per cent of the people living in Africa depend on medicinal plants and animal based medicines to satisfy their healthcare requirements [WHO, 2000]. Nauclea latifolia (family Rubiaceae) is a valuable medicinal plant that is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. It is known as African peach and may be used for traditional medicinal practices of the East and West African sub-regions of continental Africa [Dalzeil, 1957]; where various extracts of

the plant are used for the therapeutic management of malaria [Gamaniel et al, 1995]; hypertension [Akubue et al, 1998]; prolonged menstrual flow [Elujoba, 1995]; cough, gonorrhoea, stomach disorders, dysentery, ulcers and liver ailments [Traore et al, 2000]. The use of the plant in most of these conditions had been scientifically investigated and validated in studies that utilize various parts of the plant. For instance, the cardiovascular, spasmolytic, anti-plasmodial and anti-parasitic effects have been reported in studies that used various laboratory models [Marshall et al, 2000]. Laboratory studies have also provided evidence for possible sedative activities of *Nauclea latifolia* [Amos et al, 2005]. Previous studies had suggested that the leaf of *Nauclea latifolia* possesses an anti-hypertensive effect [Akpanabiatu et al, 2005; Nwosu et al, 2008].

Hypertension is a complex condition whose root cause is largely unknown and is rarely accompanied by any symptoms, therefore making it difficult to manage. As of 2000, nearly one billion people or approximately 26% of the adult population of the world had hypertension [Keamey et al, 2004, 2005]. 7.1 million Deaths per year may be attributable to hypertension [World Health Report, 2002]. It is common in both developed and developing countries [Keamey et al, 2004]. However, rates vary markedly in different regions with rates as low as 3.4% (men) and 6.8% (women) in rural India and as high as 68.9% (men) and 72.5% (women) in Poland [Keamey et al, 2005]. Hypertension is an intermittent or sustained elevation in systolic blood pressure (above 110 mm Hg) or diastolic blood pressure (above 70 mm Hg) or a systolic and diastolic pressure 20 mm Hg above the individual's baseline pressure [Chobanian et al, 2003; Odey et al, 2012]. High blood pressure is said to be present if it is persistently at or above 140/90 mmHg. This requires the heart to work harder than normal to circulate blood through the blood vessels. Hypertension can be described into two types; Primary and secondary. Primary hypertension is a type of high blood pressure with no obvious underlying medical cause while secondary hypertension are caused by other conditions affecting the kidneys, arteries, heart or endocrine system. Although mild to moderate hypertension is usually asymptomatic, accelerated hypertension is associated with headache, somnolence, confusion visual disturbances and nausea and vomiting [Singer and Kite, 2008]. Despite the fact that our understanding of the pathophysiology of an elevated arterial pressure has increased in 90% to 95% of cases, the etiology (and thus potentially the prevention or cure) is still largely unknown. Consequently, in most cases, the hypertension is treated non- specifically resulting in a large number of minor side effects and a relatively high non- compliance rate [Lackland and Egan, 2007]. Although the etiology of hypertension is directly unknown, there are many risk factors such as sedentary lifestyle, obesity, (more than 85% of cases occur in those with a body mass index greater than 25), salt (sodium) sensitivity, alcohol intake, and vitamin D deficiency. It is also related to aging and some inherited genetic mutations [Lee et al, 2008; Tuohimaa, 2009]. Family history increases the risk of developing hypertension [Bagby, 2007]. Renin elevation is another risk factor. Renin is an enzyme secreted by the juxtaglomerular apparatus of the kidney and linked with aldosterone in a negative feedback loop [Giacchetti et al, 2009]. Also sympathetic over-activity is implicated [Bailey et al, 2008]. Insulin resistance which is a component of metabolic syndrome is also thought to cause hypertension. According to [Decker et al, 2006], low birth weight has recently been questioned as a risk factor for adult essential hypertension. Hypertension is a major risk factor for stroke, myocardial infarction (heart attacks), heart failure, aneurysms of the arteries (e.g. aortic aneurysm), peripheral arterial disease and is a cause of chronic kidney disease. Even moderate elevation of arterial blood pressure is associated with a shortened life expectancy [Chobanian et al, 2003; Omodamiro and Nwankwo, 2013]. Several classes of medications, collectively referred to as antihypertensive drugs, are currently available for treating hypertension. The majority of people require more than one drug to control their hypertension. Joint National Committee on High Blood Pressure, advocates starting treatment with two drugs when blood pressure is >20 mmHg above systolic or >10 mmHg above diastolic targets. Preferred combinations are renin–angiotensin system inhibitors and calcium channel blockers, or renin–angiotensin system inhibitors and diuretics [Sever and Messerli, 2007; Suman et al, 2013]. Acceptable combinations include calcium channel blockers and diuretics, beta-blockers and diuretics, dihydropyridine calcium channel blockers and beta-blockers, or dihydropyridine calcium channel blockers with either verapamil or diltiazem.

MATERIALS AND METHODS

Chemicals

Biochemical assay kits used in this analysis were obtained from DIALAB Production and Vertrieb Von Chemisch-technischen Produkten und Laborinstumenten Gesellschaff M. B. H, A-1160 Wien-Panikengasse. Other chemicals include; *N*ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), obtained from Cayman Chemical Company Inc, 1180 East Ellsworth Road, Ann Arbor, ML 48108, USA. Captopril, obtained from Unical Pharmacy, University of Calabar, Calabar, Cross River State-Nigeria. Chloroform, Dimethyl sulphoxide, Normal saline and Distilled water.

Collection and Preparation of Plant Materials

The stem of pin cushion tree (*Nauclea latifolia*) was collected from the Teaching Hospital premises of the University of Calabar, Calabar in Cross River State-Nigeria. The plant was authenticated by the Department of Botany, Faculty of Sciences, University of Calabar. The plant part was washed thoroughly with tap water and then rinsed with distilled water. The bark was divested and chopped into small pieces and dried under shade. They were blended into fine powder using a Q-link electrical blender Model QBL-18L40. Three hundred and ten point eight grams (310.8g) of the blended stem bark was soaked in 1200ml of ethyl alcohol (80% BDH) and agitated, then allowed to stay in refrigerator for 48 hours at 4^{o}C. The mixture was first filtered with cheese cloth, then with WhatMan No 1 filter paper (24cm). The filtrate was concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37^{o} C - 40^{o}C. This was concentrated to complete dryness in water bath, yielding 37.1g (11.96%) of the extract. The extract was stored in a refrigerator from where aliquots were reconstituted for antihypertensive screening.

Laboratory Animals

Matured male and female albino wistar rats (weighing between 150-280g) were obtained from the Animal House of the Department of Zoology University of Calabar. They were maintained with rat chow (Vital Feeds LMT) and water *ad libitum*. The animals were housed, five in a cage and were exposed to 12 hour light-dark cycle and handled according to standard protocol. After the acclimatization period of two weeks, the animals were divided into two batches; treatment1 and treatment 2. Also a standard control group received 20mg/Kg bw of a standard drug, Captopril. All the treated groups and the hypertensive control group simultaneously received 40mg/Kg bw of a hypertensive agent (*N*ω-Nitro-L-arginine methyl ester hydrochloride, L-NAME), while the normal control group received 50% Dimethylsulphoxide, for two weeks (14 days).

Determination of biochemical parameters

At the end of the treatment period, the animals were anaesthetized in chloroform vapour and the blood collected via cardiac puncture into a plane tube. The blood was allowed a clotting period of two hours and then centrifuged at 3000rpm for ten minutes, using a model 0412-1 centrifuge (Cole medical instrument co.LTD, England). The serum of the centrifuged blood was collected into a clean plane tube using a syringe, and used for the determination of serum lipid, enzymes activities and electrolyte profile. The analysis was done using kits and an AJ-1222 semi-auto Biochemistry Analyzer (Easy way medical equipments LTD, made in England).

Statistical Analysis

The data were analyzed using a one-way ANOVA (in SPSS package) and the results expressed as Mean±standard deviation. All p-values <0.05 were considered significant.

Table 1: Serum Electrolytes in Rats Treated with Crude Ethanolic Stem Bark extract of N.latifolia for 14 days.

Extract	Treatment	Na^{+}(mEq/L)	Cl^{-}(mEq/L)	K^{+}(mEq/L)
Stem extract of N. latifolia	Normal control	104.67±2.47	81.28±3.11	7.80±0.33
	Hypertensive control	159.04±59.12	76.31±3.36	6.17±0.39
	Treatment 1 (150mg/kg)	116.01±30.60	97.78±2.12	4.87±0.34
	Treatment 2 (300mg/kg)	116.76±0.27	88.74±1.69	6.67±0.20
	Standard treated (20mg/kg) Captopril	113.43±0.00	71.72±3.79	6.95±0.10

Table 2: Serum lipid profile in rats Treated with Crude Ethanolic Stem Bark of Nauclea latifolia for 14 days

Extract	Treatment	T-Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
Stem Bark extract of N. latifolia	Normal control	65.13±6.40	90.58±7.96	21.52±3.32	19.63±3.32*	3.93±0.66
	Hypertensive control	77.69±19.80	106.24±11.98	14.27±0.82	30.22±2.97	5.84±0.59
	Treatment 1 (150mg/kg)	66.07±9.61*	98.95±8.74*	22.30±3.39*	24.88±2.96*	7.58±0.59*
	Treatment 2 (300mg/kg)	65.22±8.06*	96.37±8.75	20.37±8.75	25.20±2.13	6.04±0.43
	Standard treated (20mg/kg) Captopril	70.17±1.84	95.03±2.67	22.47±3.97*	25.98±0.82	6.39±0.17

Table 3: Aminotransferase Enzyme Activities In Rats Treated With Crude Ethanolic Stem Bark Extract Of *Nauclea Latifolia* For 14 Days

EXTRACT	PARAMETER	Normal control	Hypertensive control	Treatment 1 (150mg/kg)	Treatment 2 (300mg/kg)	Standard treatment (20mg/kg) Captopril
STEM EXTRACT	AST	18.55±7.18	20.40±17.45	19.20±68.75	18.70±12.25	18.60±12.10
	ALT	16.75±9.17	17.40±7.71	18.10±4.00	17.00±8.72	16.95±6.15

RESULTS

The results of the electrolyte profile and serum lipid parameters of the antihypertensive properties of the stem bark of Nauclea latifolia is presented in tables 1 and 2 below, While that of the serum enzyme activities is presented in table 3.

The animals fed with the stem bark extract had the Sodium electrolyte level of the normal (104.67±2.47) to be insignificantly lower (P>0.05) than those of treatment 1 (116.01±30.60), treatment 2 (116.76±0.27) and standard/Captopril treated (113.43±0.00) but significantly lower (P<0.05) than that of the hypertensive control (159.04±59.12). Also, the Sodium electrolyte levels of the treated were significantly lower (P<0.05) than that of the hypertensive control. This showed that the extract had a Sodium lowering effects on the animals. The Chloride electrolyte levels took a different trend, with that of the normal (81.28±3.11) being insignificantly higher (P>0.05) than those of hypertensive control and standard/Captopril treated (76.31±3.36 and 71.72±3.79 respectively) and insignificantly lower than those of treatment 1 and treatment 2 (97.78±2.12 and 88.74±1.69 respectively). This trend is however not of any serious consequence since there was a lowering effect on the Sodium electrolyte levels. Similarly, the Potassium electrolyte level for the hypertensive control (6.17±0.39) was insignificantly lower (P>0.05) than those of normal, treatment 2 and standard/Captopril treated (7.80±0.33, 6.67±0.20 and 6.95±0.10) but insignificantly higher than that of treatment 1 (4.87±0.34). These trends however showed that Sodium electrolyte being the major extra cellular electrolyte was most implicated in hypertensive condition. The Total Cholesterol levels (mg/dl) of treatment 1 (67.98±14.86) and treatment 2 (64.10±9.23) were significantly lower ($p < 0.05$) than the hypertensive control (77.69±19.80), but the decrease in normal control (65.13±6.40) and standard treatment (70.17±1.84) were not significant ($p > 0.05$). The Triacylglycerol level (mg/dl) for treatment 2 (93.21±2.57) was significantly lower ($p < 0.05$) than the hypertensive control (106.24±11.98), while the decrease in others were not significant. The higher density lipoprotein levels (mg/dl) for normal control, treatment 1, treatment 2 and standard treatment (21.52±3.32, 19.41±1.22, 18.21±2.57 and 22.47±3.97 respective) were significantly higher ($p < 0.05$) than that of the hypertensive control (14.27±0.82). This trend of changes in the lipid profile, especially total cholesterol and high density lipoprotein showed that these lipids are most implicated in hypertensive management, and the extracts have antihypertensive potentials.

The Aspartate aminotransferase levels in the treatment groups (19.20±68.75) treatment 1, (18.70±12.25) treatment 2 and (18.60±12.10) standardard/ Captopril treatment were slightly higher than the normal control (18.55±7.18) but lower than the hypertensive control (20.40±17.45). However, these changes were not significant at p<0.05. Similarly the Alanine aminotransferase levels for treatments 1, 2 and standard/Captopril treated (18.10±4.00, 17.00±8.72 and 16.95±6.15 respectively) were insignificantly higher (p<0.05) than the normal control (16.75±9.17). However, the treatment 1 was higher than the hypertensive control (17.40±7.71), while treatment 2 and standard treatment were lower. These changes are equally not significant (p<0.05). This is a possible indication that these enzymes activities are probably not directly associated with pathology.

DISCUSSION

Serum electrolytes level are some of the most commonly used biochemical indices for the assessment of hypertension [Decker et al, 2006; Vasudevan, and Sreekumari, 2007]. Sodium is the major extracellular electrolyte implicated in hypertension, while Potassium functions in collaboration with other electrolytes such as Calcium and Magnesium for the maintenance of body's homeostasis [Vasudevan, and Sreekumari, 2007]. Extra cellular Sodium electrolyte level is responsible for the extent to which vessel walls contract [Hall and Guyton, 2006]. When the Sodium level is high, there is increased contraction of the blood vessels (especially in the kidney), and hence a greater force is required to pump blood, with a consequent hypertension [Vasudevan, and Sreekumari, 2007]. This study revealed an elevation in Sodium level in the hypertensive control group, but a decrease in Chloride level. However, there was no alteration in the Potassium levels. This is in agreement with [Hall and Guyton, 2006; Odey et

al, 2012]; that the electrolyte alteration in hypertension is not directly link to Potassium. This elevation was later ameliorated by the administration of stem bark extract of *Nauclea latifolia*, and the standard drug, Captopril. This confirms the work by [Odey et al, 2012; Alcocer and Cueto, 2008]; which reported on the anti-hypertensive property of *Nauclea latifolia*. The Potassium and Chloride levels for the controls and treated were within normal range. This may be due to the fact that the extracts have cellular protection properties, with a normal extracellular Potassium level, or it may be due to the fact that these electrolytes are not directly associated with the development of hypertension [Vasudevan, and Sreekumari, 2007]. Also, the decrease in Chloride levels caused by the extracts is probably not dose dependent. Also, the extracts showed antihypertensive properties as they were able to considerably lower the Sodium electrolyte levels in hypertensive animals, compared to the controls.

The serum lipid levels are very good hypertensive markers. High levels of Cholesterol and Low Density Lipoproteins have been implicated as causatives for hypertension, as they are directly involved in artherogenicity, while High Density Lipoprotein is noted for its ability to prevent hypertension as it functions as anti-atherogenic lipid [Odey et al, 2012; Chatterjea, and Shinde, 2007; Vasudevan, and Sreekumari, 2007]. In this study, there was an increase in all the parameters under study, except HDL which suffered a decrease. The increase produced by the extract (treatments 1 and 2) were non-significant. Also the increase produced by the extract probably had no effect on the TG, LDL and VLDL levels, while there was a significant increase in the HDL levels. This reveals the fact that the phytochemical constituents of the extracts, especially Alkaloids have the tendency to ameliorate hypertensive or hyperlipidemic conditions. Hyperlipidemia is directly related to high cholesterol level in the blood [Vasudevan, and Sreekumari, 2007; Sada et al, 2013]. This has a consequent occlusion of the vessel wall, with a resultant increase in the force required to pump blood through and hence, a hypertensive condition. For the extracts to decrease the total cholesterol levels of the blood, it shows that it has anti-hyperlipidemic properties. A finding previously reported by [El-Mahmood et al, 2008]. Also, the low levels of Triacylglycerol and low density lipoprotein showed that the extracts, with the standard drug (Captopril), have the tendency to ameliorate hypertension. This property might be directly attributed to the phytochemical constituent of the extracts or a secondary mechanism that triggers other metabolic mechanisms to elicit this function.

The enzymes activities were not significantly altered. This might be due to the fact that the pathological condition was not directly associated with tissue destruction at sub-chronic stage, being that these enzymes are intracellular enzymes, and will only be found in appreciably high levels in cases of mild to severe tissue destruction [Odey et al, 2012; Chatterjea, and Shinde, 2007; Vasudevan, and Sreekumari, 2007]. Hence the induction of hypertensive condition, treatment of this condition with Nauclea latifolia stem bark extract in experimental rat model did not cause any significant change in the Aspartate aminotransferase and Alanine aminotransferase activities.

REFERENCES

[1]. Akpanabiatu MI, Umoh IB, Udosen EO, Udoh AE, Edet EE. *Indian Journal of Clinical Biochemistry* **2005**; 20 (2) 29-34.
[2]. Akubue PI, Mittal GC. *Journal of Ethnopharmacology* **1982**;*6*(4) 355–359.
[3]. Alcocer L, Cueto L. *Therapeutic Advances in Cardiovascular Disease* **2008**;*2* (3) 147–55.
[4]. Amos S, Abbah J, Chindo B, Edmond I, Binda L, Adzu B, Buhari S, Odutola A.A, WambebeC, Gamaniel KS. *Journal of Ethnopharmacology* **2005***;*10(1) 53–57.
[5]. Bagby, S.P, *Journal of Nutrition*, **2007**, 137(4)1066-72.
[6]. Bailey, M.A., Paterson, J.M. &Hadoke, P.W, *Journal of the American Society of Nephrology*, **2008**, 19(1)47-58.
[7]. Chatterjea, M.N. & Shinde, R. (**2007**). *Textbook of medical biochemistry*. 7th dition, New Delhi: Jaypee Brothers Medical Publishers.
[8]. Chobanian AV, Bakris GL, Black HR. *Hypertension* **2003***;42* (6) 1206-52.
[9]. Dalziel JK. *The useful plants of west tropical africa*, 2nd edition. London: Crown agents **1957**. P.241-48.
[10]. Decker WW, Godwin SA, Hess EP, Lenamond CC, Jagoda AS, *Annals of Emergency Medicine*, **2006,** 47(3), 237-49.
[11]. El-Mahmood, A.M., Doughari, J.H. & Chanji, F.J. *Scientific Research and Essay*, **2008**. *3* (3)102-105.
[12]. Elujoba AAA. *Fitoterapia* **1995**;*66*(3) 239-248.
[13]. Gamaniel K, Wambebe C, Amupitan J, Hussaini IM, Amos S, Awodogan A, Gaw A, Cowan RA, O'Reilly DS, Shepard J. Clinical Biochemistry. New York:Churchill Livingstone. **1995**. P.701-09.
[14]. Giacchetti, G., Turchi, F., Boscaro, M. & Ronconi, V. *Current Vascular Pharmacology*, **2009**, *7*(2)244-49.

[15]. Hall JE, Guyton AC. *Textbook of medical physiology*. 10th edition. Saint Louis, Mo: *Elsevier Saunders*.**2006**. *p.795-98.*
[16]. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J, *Lancet*, **2005**, 365(9455) 217–223
[17]. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. *J. Hypertens*, **2004**, 22(1) 11–19
[18]. Lackland, D.T. & Egan, B.M, *Current Hypertension Reports*, **2007**, 9(4)314 - 319.
[19]. Lee, J.H., O'Keefe, J.H., Bell, D., Hensrud, D.D. &Holick, M.F, *Journal of .American College of Cardiology*, **2008**, *52*(24)1949-56.
[20]. Marshall SJ, Russell PF, Philipson JD, Kirby GC, Warhurst DC, Weight CW. *Prostate* **2000**;*14*(1) 356–358.
[21]. Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Amaechina FC, Ayinde BA. *African Journal of Pharmacy and Pharmacology* **2008**;*2* (2) 037-041.
[22]. Odey M. O., Itam E. H., Ebong P. E., Atangwho I. J., Iwara I. A., Eyong U. E., Nnaligu I. J., Inekwe V. U., Johnson J. T., Ochigbo V., Udiba U. U., and Gauje B, *International Journal of Science and Technology*, **2012**, 2(6)382- 385.
[23]. Omodamiro O. D and Nwankwo C. I. *European Journal of Experimental Biology*, **2013,** 3(3)140-148.
[24]. Sada N. M., Tanko Y and Mabrouk M.A. *European Journal of Experimental Biology*, **2013,** 3(2)62-67.
[25]. Suman A, Gourikumar D and Mohammad S.A. *European Journal of Experimental Biology*, **2013,** 3(3)116-120.
[26]. Sever PS, Messerli FH, *Eur. Heart J*, **2007**, 32(20)2499–506
[27]. Singer DR, Kite A, *European Journal of Vascular and Endovascular Surgery*, **2008**, 35(6)701–8
[28]. Traore KF, Gasquet M, Di GC, Olivier E, Delmas F, Keita A, Doumbo O, Balansard G, Timon DP. *Phytotherapy Research* **2000**;*14*(2) 45–47.
[29]. Tuohimaa, P, *Journal of Steroid Biochemistry and Molecular Biology*, **2009**, 114(2)78-84.
[30]. Vasudevan DM, Sreekumari S. *Textbook of Biochemistry for Medical Students*. 5th Edition, New Delhi: Jaypee Brothers Medical Publishers; **2007**. P. 239-46
[31]. WHO. Classification of hypertension. Report of WHO Scientific Group. Technical Report Series **1978**; 657: 87-95.
[32]. WHO. *Expert Committee on medicinal importance of native plants*. Technical report series.
[33]. WHO Geneva, **2000**
[34]. Wong TY, McIntosh R. British Medical Bulletin 2005; *73*(74) 57–70.
[35]. World Health Report, http:// www.who.int/whr/ **2002**.