Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.6, 2016

Effects of Herbal "Gadagi" Tea on Some Cardiovascular Risk Factors in Experimental Rats

*Ibrahim, A., Imam I. U., Abubakar S. M., Murtala, Y., Auwal, S.M. and Babandi, A.
Department of Biochemistry, Bayero University, PMB 3011, Kano-Nigeria

Abstract
Cardiovascular disease (CVD) remains a major cause of mortality worldwide in spite of the recent advances in medical and surgical treatment. Twenty Wistar albino rats were divided into Four (4) equal groups each comprising of five (5) members. The groups (A, B & C) were orally administered with prepared Herbal/Garlic "*Gadagi*" Tea, at low dose (3 mg/kg), standard dose (6 mg/kg) and high dose (12 mg/kg) respectively. Group D served as normal control. After two weeks of Herbal/Garlic "*Gadagi*" tea administration, all the 20 Wistar albino rats were sacrificed by decapitation and their blood samples were collected and used for biochemical analyses. Total Cholesterol (TC), HDL-cholesterol, LDL-Cholesterol, Triglycerides, Sodium, Potassium, AST, CK, and LDH were analysed using standard methods. There was a significant increase ($P < 0.05$) in the levels of TC, LDL-Cholesterol, Potassium and CK in group C compared to control and significant decrease ($P<0.05$) in HDL-Cholesterol in group A compared to normal. Dose- dependent increases were observed ($P<0.05$) in TC, LDL-cholesterol, Triglycerides and LDH at 6mg/kg and 12mg/kg doses; Potassium at 3mg/kg and 6mg/kg doses and at 6mg/kg and 12mg/kg doses; CK at 3mg/kg and 6mg/kg doses respectively. Generally, the current research suggests that herbal/Garlic "*Gadagi*" tea might be a risk factor of cardiovascular diseases, and may be toxic to both the heart and peripheral vascular tissues at doses beyond 6 mg/kg.
Keywords: *Gadagi*, cardiovascular diseases, herbal medicine, tissue damage markers

1.0 Introduction

Cardiovascular diseases include coronary heart diseases or coronary artery diseases, cardiomyopathy, heart failure, endocarditis, cerebrovascular disease and peripheral arterial diseases among others. Almost all cardiovascular diseases in a population can be explained in terms of a limited number of risk factors: age, gender, high blood pressure, high serum cholesterol level, tobacco smoking, excessive alcohol consumption, family history, obesity and lack of physical activity, psychosocial factors, diabetes mellitus and air pollution (Bridget and Kelly, 2010; WHO, 2012). While the individual contribution of each risk factor varies between different communities or ethnic groups, the consistency of the overall contributions of these risk factors is remarkably strong (Yusuf *et al.,* 2004). Some of these risk factors, such as age, gender or family history are immutable, however many important cardiovascular risk factors are modifiable by life style change, drug treatment or social change (Martin, 2006).

While the causes of cardiovascular diseases are diverse, atherosclerosis and/or hypertension are the most common. Cardiovascular diseases are the leading cause of death and disability in the world (WHO, 2012). Although a large proportion of cardiovascular diseases are preventable, they continue to rise mainly because preventive measures are inadequate (WHO, 2012).

"*Gadagi*" (meaning energy in ***Hausa***) is a type of tea that is consumed mostly in the northern part of Nigeria. The tea consumption dates back 65 years with consumption in few spots. It is still a new entrant as a stimulant. Its preparation is not radically different from the way normal tea is prepared. It is a mixture of sugar and tea of highland brand boiled in water with some plants such as African mahogany (*Khaya senegalensis*) lemon grass (*Cymbopogon citratus*) and mint plant (*Mentha palustris*). It is consumed as a hot beverage mostly by drivers and commercial motorcyclists in Northern Nigeria and more particularly in Kano state (Atiku *et al*., 2009). Other silent users are tailors and labourers involved in strenuous physical jobs. Those who use it believe that, it can increase their power of endurance and their ability to forego food and sleep. It is also believed to be a source of energy probably due to its enriched sugar content. There are three major types of "*Gadagi*" tea, viz: "*Sak*", "*Sada*" and "*Magani*". Some of the users add some drugs such as *Alabukun* (acetylsalicylic acid), marijuana and buta(butazolidin) to enrich the "*Gadagi*" tea (Atiku *et al*., 2009).

Despite the fact that "*Gadagi*" tea is being consumed indiscriminately, there is little or no scientific evidence to show that its consumption is safer or has no effect on the heart tissues. Individual parts of different plants used in herbal "*Gadagi*" tea preparation were known to have certain medicinal value, but synergistic effect of multiple herbal "*Gadagi*" tea concoction on cardiovascular risk factors remains unknown. However, an estimated 17.3 million people died from cardiovascular diseases in 2008; over 80% of CVDs death takes place in low and middle income countries(Nigeria inclusive); and by 2030, almost 23.6 million people will die from CVDs. Hence, investigation of herbal "*Gadagi*" tea effects on cardiovascular risk factors would be of significance to humanity, as it may positively or negatively influence the pre-estimated mortality and disability by World Health Organisation.

2.0 Materials and Methods

2.1 Materials:

- Randox brand Commercially Prepared Reagent Kits for T. Cholesterol, Triglcerides, HDL-cholesterol and AST obtained from *Randox Laboratories Ltd., 55 Diamond Road, crumlin, Co. Antrim, United Kindom, BT294QY.*
- AGAPPE brand Commercially Prepared Reagent Kits for C.K and LDH-p obtained from Agappe *Diagnostics, Switzerland, GmbH.*

2.2 Preparation of Herbal/Garlic *Gadagi* tea

All the components of the Herbal/Garlic "*Gadagi*" tea were sourced from *Kurmi* market with exception of the Red Tea leaves, which was obtained from *Igbo* road, "*sabon gari*" market of Kano, Kano state - Nigeria. The components of the Herbal/Garlic "*Gadagi*" tea were powdered using metal pestle and mortar and then sieved. The proportional quantity of each component was then weighed and then put in a clean stainless steel container. A litre of distilled water was then added to the mixture and then boiled for an hour in a tightly closed container to minimize steam escape.

The relative proportional quantities of the components are:

- *Allium sativum* (Garlic) 20.0g (10.52%)
- *Mentha palutris*(Mint Plant) 3.50g (1.84%)
- *Cymbopogon citratus* (Lemon Grass) 4.0g (2.11%)
- *Zingiber officinale* (Ginger) 3.50g (1.84%)
- *Eugenia caryophyllus* (Clove) 1.5g (0.79%)
- *Piper guineense* (West African Pepper) 1.5g (0.79%)
- *Aspalathus linearis* (Red Tea Leaves) 4.0g (2.11%)
- *Xylopia aetiopica* (Grain of Selim) 2.0g (1.05%)
- Sugar 150.0g (78.9%)
- Distilled water 1.0 litre

2.3 Acquisition and Care of experimental animals

Twenty (20) albino rats weighing between 100-120g were obtained from the animal house of the Department of Biological Sciences, Bayero University, Kano. They were allowed food and water *ad-libitum* and exposed to twelve hour light-dark cycles and the experiments conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996). The animals were also handled according to international guidelines, i.e. the Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG407) (OECD, 2006).

Methods

2.4 Experimental design

The animals were divided into four (4) groups of five (5) animals each. Three of the groups were administered orally with the Herbal/Garlic "*Gadagi*" Tea, by intubation, at low dose (3mg/Kg- Group A), standard dose (6mg/Kg- Group B) and high dose (12mg/Kg- Group C) respectively for two weeks, whilst one group served as control (given only water – the vehicle). All the rats were allowed free access to food and water during the course of the experiment. Volume of "*Gadagi*" tea administered as the standard dose was determined based on the average weight of the rats in relation to 70kg man that normally consumes 700cm^3.

After two weeks of administrations, all the 20 rats were sacrificed by decapitation and their blood samples collected separately into clean test tubes and centrifuged at 1000 revolution per minute for ten minutes. Serum was obtained and used for biochemical analyses instantly.

2.5 Methods

Total cholesterol, TGS, LDL-cholesterol and HDL-cholesterol were determined using Randox commercially prepared kits; Na and K were determined using flame photometry; Aspartate Transaminase Determination (Reitman *et al.*, 1957) Method. Lactate dehydrogenase Determination (Wei-Bharr Method, 1975) Creatine Kinase Determination using Trendelenburg (1982) Method.

3.0 Results and Discussion

The mean serum total cholesterol, LDL-cholesterol, and triglycerides showed a dose- dependent increase in experimental groups (standard dose and high dose), while HDL-cholesterol showed a decreasing pattern as the dosage increases, suggesting an inverse relationship. Dose-dependent increase was also observed in serum sodium level in experimental rats, though no significant difference was found in all the groups. However, the serum level of potassium decreases significantly ($p<0.05$), as the dosage increases- suggesting an inverse relationship. There

is general increase in mean serum cardiac enzyme activity in the test groups. Both AST and LDH activity increase significantly as the dosage increases ($p<0.05$).

There was a significant increase ($P < 0.05$) in the levels of T. Cholesterol, LDL-Cholesterol, Potassium and CK in group C compared to control and significant decrease ($P < 0.05$) in HDL-Cholesterol in group A compared to normal. Dose- dependent increases were observed ($P < 0.05$) in T. Cholesterol at 6mg/kg and 12mg/kg doses; LDL-cholesterol at 6mg/kg and 12mg/kg doses, Triglyceride at 6mg/kg and 12mg/kg doses, Potassium at 3mg/kg and 6mg/kg doses and at 6mg/kg and 12mg/kg doses; CK at 3mg/kg and 6mg/kg doses; LDH at 6mg/kg and 12mg/kg doses respectively.

Table 1: Effect Of Different Doses Of Herbal "*Gadagi*" Tea On Lipid Profile In Experimental Wistar Albino Rats

	T.Chol (mmol/L)	%Δ	HDL-Chol (mmol/L)	%Δ	LDL-Chol (mmol/L)	%Δ	Trig(mmol/L)	%Δ
CONTROL (0 mg/Kg)	5.06^{a} ± 0.99	0	1.42^{c} ± 0.15	0	2.97^{d} ± 0.60	0	1.46 ± 0.73	0
LOW DOSE (3 mg/Kg)	4.58 ± 0.84	-10	1.08^{c} ± 0.31	-24	3.10 ± 0.82	4	1.68 ± 0.24	15
STANDARD DOSE (6 mg/Kg)	5.72^{b} ± 1.70	13	1.12 ± 0.13	-21	3.87^{c} ± 1.43	30	1.79^{b} ± 0.31	22
HIGH DOSE (12 mg/Kg)	$6.98^{a,b}$ ± 1.24	38	0.94 ± 0.46	-34	$5.11^{d,c}$ ± 0.90	72	2.04^{b} ± 0.05	32

Result are expressed as Mean ± S.D. n= 5. Values bearing similar superscripts in the same column are significantly different at $P < 0.05$ compared to each other.

Table 2 : Effect Of Different Doses Of Herbal "*Gadagi*" Tea On Electrolytes In Experimental Wistar Albino Rats

	Sodium (mg/L)	Potassium (mg/L)
CONTROL (0 mg/Kg)	135.06 ± 5.52	4.92^{a} ± 1.50
LOW DOSE (3 mg/Kg)	138.60 ± 3.45	3.26^{b} ± 0.65
STANDARD DOSE (6 mg/Kg)	140.18 ± 22.50	$3.06^{b,c}$ ± 0.65
HIGH DOSE (12 mg/Kg)	154.88 ± 22.37	$2.77^{a,c}$ ± 0.93

Result are expressed as Mean ± S.D. n= 5. Values bearing similar superscripts in the same column are significantly different at $P < 0.05$ compared to each other.

Table 3 : Effect Of Different Doses Of Herbal "*Gadagi*" Tea On Some Cardiac Enzymes In Experimental Wistar Albino Rats

	AST (U/L)	CK (U/L)	LDH (U/L)
CONTROL (0 mg/Kg)	10.20^{b} ± 3.11	$89.79^{a,c}$ ± 25.04	73.43 ± 43.89
LOW DOSE (3 mg/Kg)	11.40^{b} ± 5.37	$94.10^{c,d}$ ± 12.13	65.19 ± 18.28
STANDARD DOSE (6 mg/Kg)	22.60 ± 11.19	193.23^{d} ± 62.99	81.75^{c} ± 24.16
HIGH DOSE (12 mg/Kg)	38.60 ± 28.59	163.43^{a} ± 64.00	177.40^{c} ± 98.63

Result are expressed as Mean ± S.D. n= 5. Values bearing similar superscripts in the same column are significantly different at $P < 0.05$ compared to each other.

4.0 Discussion

Several studies have consistently indicated correlation between serum levels of cholesterol, TG, HDL- and LDL-cholesterols with hypertension (O'Brien *et al.,* 2007; Grossman and Messerli, 2012). The observation in this work may likely be due to the fact that the mechanism involving lipid alteration may have been disturbed by the administration of Herbal "*Gadagi*" tea.

A study on garlic indicated that it prevents hypertension by chronic inhibition of nitric oxide synthesis without altering blood pressure in garlic fed rats. This indicates that though the base line of measured parameter remains the same, there may be a change in situation when there is hypertension (Pedraza *et al.,* 1998). Low level of LDL-cholesterol is known to correlate with a low incidence of coronary heart disease, particularly arteriosclerosis. Increase of 1% cholesterol is reported to have resulted in a 3% increase in coronary heart disease. Equally a reduction in LDL-cholesterol by 2 mg/dl can result in 1% reduction in the risk for coronary artery disease (Lipid research clinics programme, 1984). The significantly high LDL-cholesterol (30% -72%) obtained in this work suggests the possibility of herbal "*Gadagi*" tea to have no ameliorating effect on cardiovascular conditions but rather a high risk factor which is in agreement with the work of Howard *et al*. (2000) where it was reported that "a 10mg/dL increase in LDL-cholesterol was associated with a 12% increase in CVD risk."

However, Na^{+}- K^{+} *ATPase* may be affected since this enzyme is required for the movement of these electrolytes across the membrane. The serum levels of K^{+} and Na^{+} were determined and there was no significant difference ($P > 0.05$) in the Na^{+} level but K^{+} level decreases significantly ($P < 0.05$). Potassium, which is in the intra-cellular fluid, has been reported to be among the protective electrolytes against hypertension (Nurminen *et al.,* 1998; Ascherio *et al*., 1998).

Tissue enzyme activity as well as cholesterol and triglyceride concentrations in different animals' species have been extensively investigated by several workers (Clampitt and Hart, 1978; El-Newechy *et al.,* 2002; Oluba *et al.,* 2009).

The serum cardiac enzymes detected suggest possible damages to the cardiovascular tissues which may be attributed to the relatively low level of HDL-cholesterol and higher level of LDL-cholesterol- which posed the animals to the genesis of atherosclerosis. Strong correlations have been shown between increased plasma total cholesterol, LDL-cholesterol and increased incidence of coronary heart disease (Edionwe and Kies, 2001; Kamisah *et al.,* 2005). Atheroslerosis, which involves deposits of fatty substances, cellular waste products, calcium and fibrin, is the leading cause of illness and death in the United States and most other western countries (Ross, 1999). HDL cholesterol has a protective effect against cardiovascular disease as it removes excess cholesterol from circulation and carries it back to the liver where it is degraded or converted into bile acids (Ahmad *et al.,*1992).

The dose-dependent increase in the lipid profile suggests impairment in lipid transport or LDL-receptor deficiency, which plays role in the genesis of the associated hypercholesterolemia. The lipid raising effect of some trado -medicinal plants have been reported (Sundram *et al.,*1999; Emenalom *et al.,*2004).Some workers had earlier observed increase in serum cholesterol on administration of crude drug extract; Ahmad *et al.* (1992), observed an increase in serum cholesterol levels on administration of aqueous extract of *Aplotaxis lappa.* Another work has

also demonstrated the ability of *H.rosa-sinensis* to influence liver metabolism towards increased synthesis of lipids (Kate, 2010). This effect is however, dose dependent- suggesting that the use of *H. rosa-sinensis* in alternative or complementary medicine should not exceed the 500 mg/kg dose level (Kate, 2010). In this study, it should thus be noted that the use of "*Gadagi*" tea should not exceed the low dose (3 mg/Kg) in rats.

Evaluation of blood total cholesterol concentrations and other lipid abnormalities are part of a number of risk factors identified for cardiovascular diseases (CVD) (Bell, 2000). Cardiovascular disease is the dominant single cause of premature mortality in the world (Anand *et al*., 2000). The effect of dietary changes on serum lipid levels differs significantly between individuals and species. Humans and animals however show a certain consistency in the response of their serum lipids to fat-modified diets (Desroches and Lamarche, 2004). The difference in response may be caused by variation in genes regulating serum lipid levels (Clifton and Abbey, 1997). Lactate dehydrogenase (LDH), alanine amino- transferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltranspeptidase (γ -GT) in addition to cholesterol and triglyceride concentrations are demonstrated to be associated with cardiovascular risk factors (Conigrave *et al.,* 1993; Amdt *et al.,* 1998; Whitefield, 2001) as has been the case in the current study.

In a study (Nwinuka *et al.,* 2005), Proximate composition and levels of some toxicants (anti-nutrients) in four commonly consumed spices reveals the presence of (30.05 ± 0.15 mg) cyanogenic glycosides per 100g of Ginger; (37.06 ± 0.05 mg) per 100g of Garlic and (31.80 ± 0.03 mg) per 100g of West African pepper- which are both components of herbal "*Gadagi*" tea and may be attributed to the "toxicity" observed in this study.

5.0 Conclusion

The result of this work appears to be contradictory to the earlier report that indicated plants used in "*Gadagi*" tea preparation as important medicinal agent. This contradiction can be attributed to the fact that, at each plant level the medicinal property can be manifested, but their combined effects in "*Gadagi*" tea may lead to damages to cardiovascular tissues. This may be linked to the mode of preparation of the tea or quantity of the plants used in preparation of the tea. The curative properties of most plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and sterols and also the elemental composition. It is plausible to suggest that these bioactive constituents of plants involved in herbal "*Gadagi*" tea may have lost their native biomedicinal property or they strongly favored lipogenesis. This fact must be considered by the "*Gadagi*" tea sellers and/or consumers from both the standpoint of preparation and dosage. Finally, the overall result of this work suggests that herbal "*Gadagi*" tea is toxic to the Heart and peripheral vascular tissues.

Acknowledgments

We are grateful to the entire department of Biochemistry, Bayero University, Kano-Nigeria, for their support and contributions.

References

Ahmad, M., Saeed, M.M., Alam, H. and Ashgar, Z. (1992). Biological studies of indigenous medicinal plants II: Effects of *Aplotaxis lappa* Dcne on various parameters of liver metabolism in rabbits. *J. Islam Acad. Sci.* 5 : 61- 66.

Amdt, V., Brenner, H., Rothenbacher, D., Zschenderlein, B., Fraisse, E., Fliedner, T.M. (1998).Elevated liver enzyme activity in construction workers.Prevalence and impact on early retirement and all-cause mortality.*Int. Arch. Occup. Environ. Health*, 71: 405-412.

Anand, S., Yusuf, S., Vuksan, V., Devenesen, S., Teo, K.T., Mortaque, P.A., Kelemen, L., Yi, C., Lonn, E., Gerstein, H., Hegele, R.A., McQuenn.M. (2000). Differences in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada .The study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet,*356: 279-284.

Ascherio, A.,Rimm, E.B., Hernan, M.A., Giovannucci, EL., Kawachi, I., Stampfer, M.J.and Willettt, W.C. (1998). Intake of potassium, calcium and fiber and risk of stroke among US men. Circulation- *AHA Journals* 98:1198-1204.

Atiku, M.K., Adamu, D.J.M., Gadanya, A.M. and Shehu, M.A. (2009).The effect of "Gadagi" tea on liver function and serum glucose concentration in Albino rats. *Bayero J. of Pure and Applied Sci*. 2 (1):125-127.

Bell, D.S.H. (2000). Cardiovascular disease in South Asians. *Lancet*, 356: 1108-1116.

Bridget, B. K. (2010). *Promoting Cardiovascular Health in the Developing World*: A Critical Challenge to Achieve Global Health. Washington, D.C: National Academies Press.

Clampitt, R.B. and Hart, R.J.(1978). The tissue activity of some diagnostic enzymes in ten mammalian species. *J. Comp. Pathol.,* 88: 607-621.

Conigrave, K.M., Saunders, J.B., Reznik, R.B., Whitfield, J.B. (1993). Prediction of alcohol-related harm by laboratory test results.*Clin. Chem.,*39: 2266-2270.

Desroches, S. and Lamarche, B. (2004). Diet and low-density lipoprotein particle size.*Curr.Atheroscler. Rep*., 6:

453-460.

Edionwe, A.O. and Kies, C. (2001). Compassion of palm and mixtures of refined palm and soyabean oils on serum lipids and faecal fat and fatty acid excretion of adult humans. *Plant foods. Hum. Nutr.*56:157- 165.

El-Newechy, T.K., Al-Qarawi, A.A. and Abdel-Rahman, H.A. (2002). "Some studies on stiff lamb disease in Qassim region in Saudi Arabia.1:Enzymatic profile in free, subclinically and clinically affected lambs both before and after treatment with vitamin E and selenium preparation". *Small Rum. Res.*35: 219-223.

Emenalom, O.O., Okoli, L.C., Udedibie, A.B.L. (2004). Observations on the pathophysiology of weaner pigs fed raw and preheated Nigerian *Mucuna pruriens*, (velvet bean) seeds. *Pakistan J.*134:170- 178.

Grossman, E. and Messerli, F.H. (2012). "Drug-induced Hypertension: An Unappreciated Cause of Secondary Hypertension". *Am. J. Med.* 125 (1): 14–22.

Howard, B. V., David C. R., Maurice, L. S., Elisa, T. L., Dorothy R., Richard, B. D.,Linda, D., Cowan, R. Stuart G., Thomas, K. W., Oscar, T. G, W and James, H.(1999). LDL Cholesterol as a Strong Predictor of Coronary Heart Disease in Diabetic Individuals with Insulin Resistance and Low LDL: The Strong Heart Study. *Atbv. AHA Journal* : 830-835.

Kamisah, Y., Adam, A., Wan-Ngah, W.Z., Gapor, M.T., Azizah, O. and Merzuki A. (2005). Chronic intake of red palm oil and palm olein produced beneficial effects on plasma lipid profile in rats. *Pakistan J. of Nutr.*4(2):89-96.

Kate, E.I (2010). Tissue lipid profile of rats administered aqueous extract of *Hibiscus rosa-sinensis.* Linn*. J. of Basic and Appld. Sci.* 6(1): 1-3.

Lipid Research Clinics Programme (1984). The lipid research clinics coronary primary prevention trials results, 1: reduction in the incidence of heart disease. *Jama* 251:351-364.

Martin, A. C. (2006). *Clininical Chemistry and Meatabolic Medicine*. Published by Hodder Arnold, 7th ed. Pg 327.

Nurminen, M.L., Krepla, R. and Vapattalo, H. (1998). Dietary factors in the pathogenesis and treatment of hypertention*. Ann. Med.*30 (2):1433-1450.

Nwinuka, N.M., Ibeh, G.O. and Ekeke, G.I. (2005).Proximate composition and levels of some toxicants in four commonly consumed spices. *J. Appl. Sci. Environ. Mgt.*9 (1) 150 – 155.

O'Brien, Eoin., Beevers, D.G., Lip H. and Gregory, Y. H. (2007). *ABC of hypertension*. London: BMJ Books. ISBN 1-4051-3061-X.

Oluba, O.M., Onyeneke E.C., Ojieh, G.C. and Eidangbe, G.E. (2009). "Effect of soy protein on selected enzymes in some tissue of rats fed a cholesterol diet". *Int. J. Med. Med. Sci.*, 1(9): 400–406.

Pedraza, C.J., Tapia, E., Medina, C.D., Angeles, G. M-delos, Franco, M., Delos-Angeles-Granadous, M. (1998). Garlic prevent hypertension induced by chronic inhibition of nitric oxide synthesis. *Life Sci.* 62 (6) : 71-77.

Reitman S. and Frankel S. (1957).A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases*.Am. J. Clin. Pathol.* 28(1):56-63.

Ross, R. (1999). Atherosclerosis an inflammatory disease *.N. Engl. J.* 340:115- 126S.

Sundram, K., Khor, H.T. and Ong, A.S. (1999). "Effect of dietary palm oil and its fractions on rat plasma and high density lipoprotein". *Lipids* 25:1807-13.

Whitefield, J.B. (2001). "γ-glutamyl transferase". *Crit. Rev. Clin. Lab. Sci.,*38: 263-355.

World Health Organization(WHO). (2008). The Global Burden of Disease: 2004 Update. Geneva: World Health Organization. ISBN 92-4-156371-0.

World Health Organization(WHO). (2009). "WHO Disease & injury country estimates". Retrieved Nov. 11, 2009.

Yusuf , S., Hawken, S. and Ounpuu, S. (2004). "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study". *Lancet* 364 (9438): 937–52.