Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 43-49
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

EFFECTS OF AQUEOUS EXTRACT OF BEETROOT AND MISTLETOE ON CAFFEINE AND PARACETAMOL INDUCED HEART LESION ON SOME SERUM ENZYMES OF WISTAR RATS

S. I. Omeodu, E. C. Ezeonwumelu, B. M. Aleme and P. I. Anele

Department of Biochemistry, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.
Corresponding authors Email:ikstar2000@yahoo.com, ebubeze08@yahoo.com, benaleme@yahoo.com
Phone: 234-8035490273, 234-8146039428, 234-8035520786

Received: 10-10-16
Accepted:14-11-16

ABSTRACT
Beetroot (Beta vulgaris) is used as food and drink (sugar, root vegetable, leafy vegetable). In alternative medicine, mistletoe (Viscum album) leaves and young twigs are used by herbalists and preparations made from them are popular in Europe, especially in Germany, for treatment of circulatory and respiratory problems. Blood samples were collected by cardiac puncture into heparin bottles, fluoride oxalate bottles and put into plain bottles with 10% formalin. Plants have always been a good source of drugs. Numerous studies have shown that a wide variety of plant extracts are effective in the cardiovascular system. Caffeine + Paracetamol is a common pain relief drug widely used. The objective was to evaluate the effects of caffeine and paracetamol induced heart lesion on some serum enzymes of wistar rats and the effects of aqueous extracts of beetroot and mistletoe on the same serum enzymes after treatment.

Key words: aqueous extracts, serum enzymes, young twigs.

INTRODUCTION
For thousands of years, herbs have been used a scents, foods, flavourings, medicines, disinfectants and even as currency (Shanti *et al*., 2011).
In recent years, many people living in industrialized countries have been taking a second look at herbal medicine due to the rising cost of medicine and healthcare in their nations. There are a number of herbal systems that dominate the world today, and these systems are Chinese herbs, ayurvedic medicine, Roman and Greek herbs (Scheck *et al.*, 2006).

The major problem encountered in the administration of these plants as a remedy for an illness, is in its dosage, quality control of herbal medicine and good practices are indispensible for the advancement of herbal medicine system (Ljunberg *et al.*, 2008). Beetroot (*Beta vulgaris)* is used as food and drink (sugar, root vegetable, leafy vegetable). Mistletoe was one of the many species originally described by Linnaeus. Its species name is the Latin adjective albus 'white'. It and the other members of the genus, *Viscum* were originally classified in the mistletoe family, *Viscaceae*, but this family has since been sunk into the larger family, *Santalaceae*. Several subspecies are commonly accepted (Grubben and Denton, 2004). Mistletoe is used as flavouring, an ingredient of pomace brandy based and made in Istra, Croatia (Deeni and Sadiq, 2002). In alternative medicine, mistletoe leaves and young twigs are used by herbalists and preparations made from them are popular in Europe,

especially in Germany, for treatment of circulatory and respiratory problems (Finegold *et al*., 2013). Cardiovascular diseases (CVD) are disorders of the blood vessels and the heart (circulatory system), which encompasses a number of specific illness like; Coronary heart disease, heart failure, arrythmias, congenital heart disease, stroke, arterial and pulmonary hypertension (Ljunberg and Weitzberg, 2011). Cardiovascular diseases (CVD) remain the most prevalent cause of human morbidity and mortality all over the world (Polter *et al.*, 2008).

According to a survey by Global Burden of Disease Study, 29.6% of all deaths worldwide were caused by CVDs in 2010 (Hobbs *et al.*, 2012). There is growing evidence showing that many herbal medicines and their active ingredients contribute to the standard therapy for CVDs, for example, beetroot, one of the vegetables with the highest nitrate content (>250 mg/100g fresh weight) (Larsen, 2007).

Some people experience insomnia or sleep disruption if they consume caffeine, especially during the evening hours (Venuraju *et al.*, 2010). The study aimed at investigating the cardiovascular effects of beetroot and mistletoe. The objective was to evaluate the effects of caffeine and paracetamol induced heart lesion on some serum enzymes of wistar rats and the effects of aqueous extracts of beetroot and mistletoe on the same serum enzymes after treatment.

MATERIALS AND METHODS

Preparation of Plant extract

Fresh beetroots were obtained from fruits garden, D-line, Port Harcourt while fresh mistletoe leaves were obtained from Delta Park, just at the VC's gate of the University of Port Harcourt. They were, then, identified and kept at the herbarium of the University of Port Harcourt for reference purposes. Both plants were washed and air-dried for seven days before grinding, the beetroots were sliced in pieces for easy drying after which they were ground to powder form and then taken to the laboratory for extraction.
The powder form samples of beetroot and mistletoe were weighed 10 g each and dissolved in 100 ml of distilled water each. After vigorous shaking of the sample mixture for 10 minutes, it was allowed to stand for 24 hours. The mixture was then filtered thrice, each time through a piece of white cotton cloth and into a collecting beaker until a fine filtrate or clear solution was obtained. The clear solution become the stock. Dilutions were made as recommended.

Experimental Animals

A total of twenty five (25) Wistar albino rats (3 - 4 weeks old), weighing 100-200g were obtained from the Department of Animal and Environmental Biology, University of Port Harcourt Animal House. They were housed in stainless steel cages (5 rats per cage) and kept in a well-ventilated room.
The rats were fed with standard diet (Livestock Feeds Nig. Ltd. Ikeja, Nigeria) and water *ad libitum.* The standard guideline for the use of experimental animals (including applying humane actions during sacrifice) were adhered to.

Preparation of Sudrex Dilution

0.29mg/g of sudrex equivalent to $1^{3}/_{4}$ tablets was dissolved in 5 ml of distilled water and administered to rats weighing 175g, $1\ ^{1}/_{3}$ tablet approximately 0.25mg/g of sudrex was dissolved in 3ml of water and administered to each rat weighing 150g, $1^{1}/_{2}$ tablets dissolved in 2ml of distilled water was administered to each rat weighing 125g and 1 tablet of sudrex dissolved in 1ml of water was administered to each rat weighing 100g. The animals were fasted overnight.

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 43-49
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Table 3.1 Treatments administered to various groups

Groups	Treatments	Number of rats
Group One (normal control). Rats weighed 200 g.	Normal feed + water	5
Group Two (caffeine/paracetamol induced). Rats weighed 175 g.	Sudrex (25 ml/kg) + feed and water	5
Group Three. Rats weighed 150 g.	33 mg/g beetroot + mistletoe extracts + 20 ml/kg of sudrex + feed and water	5
Group Four. Rats weighed 125 g.	22 mg/g beetroot + mistletoe extracts + 16 ml/kg of sudrex + feed and water	5
Group five. Rats weighed 100 g.	14 mg/g beetroot + mistletoe extracts + 10 ml/kg of sudrex + feed and water	5

Animals were sacrificed after 10 days post extract treatment under chloroform anaesthesia. Blood samples were collected by cardiac puncture into (a) heparin bottles, (b) fluoride oxalate bottles and (c) put into plain bottles with 10% formalin.

Enzymes Assays

The determination of alanine aminotransferase (ALT) in the serum samples, performed at 37^0C, was determined by the colorimetric method (Ibekwe *et al.*, 2007). Alanine aminotransamine catalyses the transfer of the amino group L-Alanine and α-ketoglutarate to form pyrurate and glutamate. Creatine Kinase was determined by electrophoresis method (Monanu, 2004). For aspartate aminotransferase, L-aspartate replaced L-alanine (Ibekwe *et al.*, 2007). For the estimation of lactic acid dehydrogenase, a decrease in absorbance at 340 nm was observed (Kim *et al.*, 2001). For the determination of serum protein, the biuret method was used (Kaplan and Pesce, 2001).

Histopathology

This sections of the preserved liver slices obtained with the use of a tissue slicer were fixed on microscopic slides and stained before observing under the microscope following the method described by Baker and Silverton (2005).

Statistical analysis

All data were statistically analyzed with ANOVA at 95% confidence level and expressed as mean ± SEM.

RESULTS

The table 1 shows the mean concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactic acid dehydrogenase (LDH) and total protein (TP).

Table 1: Comparison of the liver function markers (AST, ALT, TP), CK and LDH mean concentration and SEM values for each of the five groups of rats

	AST	ALT	CK	LDH	TP
Group 1	9.00 ± 2.00	118.00 ± 2.00	10.00 ± 2.00	24.67 ± 2.00	73.00 ± 1.00
Group 2	23.33 ± 1.53	270.00 ± 2.00	30.00 ± 1.53	36.67 ± 2.00	63.00 ± 2.00
Group 3	19.33 ± 1.00	220.00 ± 1.00	22.60 ± 1.53	22.67 ± 1.15	69.00 ± 1.00
Group 4	19.33 ± 2.00	225.00 ± 7.23	24.00 ± 2.00	28.00 ± 1.00	65.00 ± 1.00
Group 5	20.66 ± 2.00	226.00 ± 5.27	24.00 ± 1.73	28.67 ± 2.00	65.00 ± 1.00

HISTOPATHOLOGY RESULTS

Microphotographing of the four heart samples revealed no visible cardiac damage. Cardiac damage appears invisible during early examination, in all the extract treated groups.

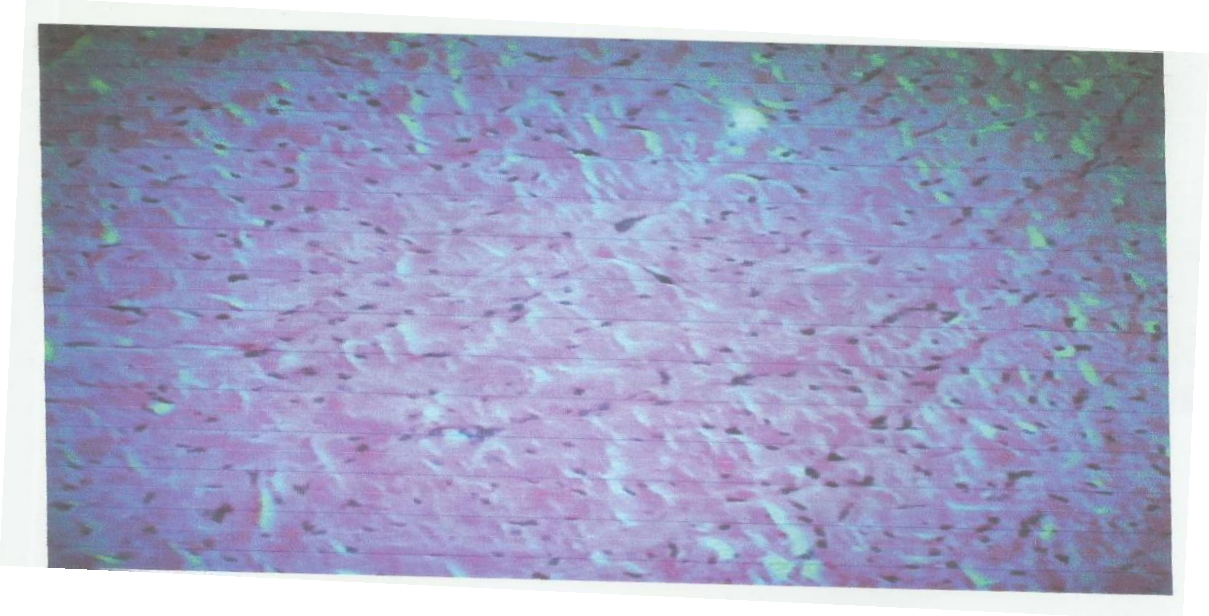

Figure 1: Microphotographing of the first rat heart sample. No visible cardiac damage. Cardiac damage appears invisible during early examination in this extract treated group (group 2) from which a rat heart sample was used. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

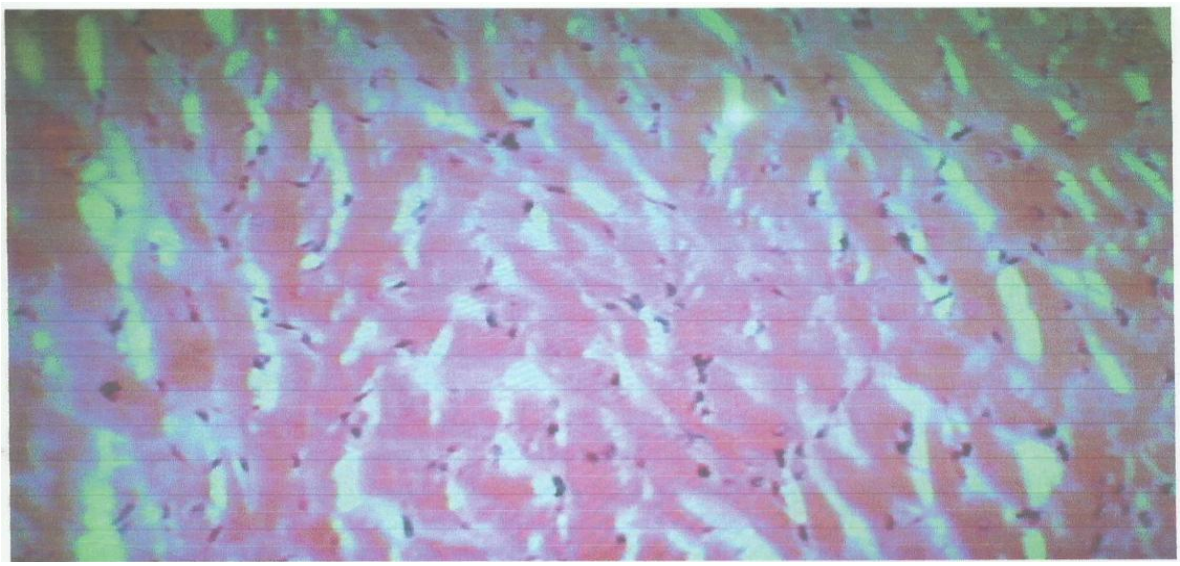

Figure 2: Microphotographing of the second rat heart sample. No visible cardiac damage. Cardiac damage appears invisible during early examination in this extract treated group (group 3) from which a rat heart sample was used. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 43-49
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

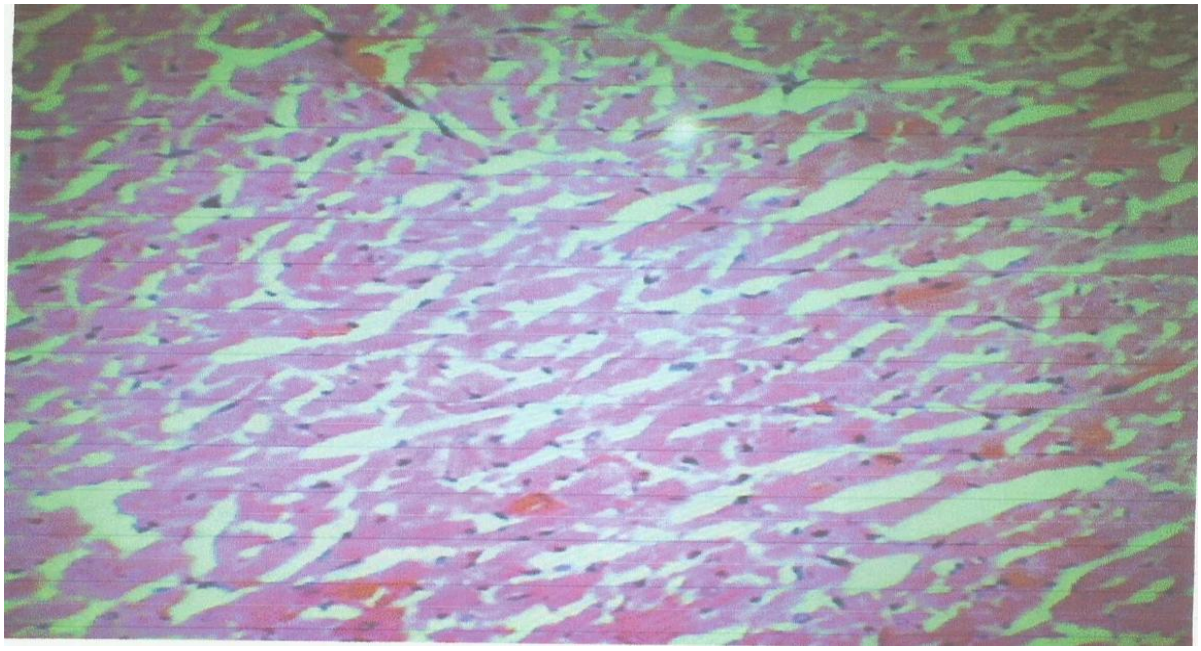

Figure 3: Microphotographing of the third rat heart sample. No visible cardiac damage.
Cardiac damage appears invisible during early examination in this extract treated group (group 4) from which a rat heart sample was used. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

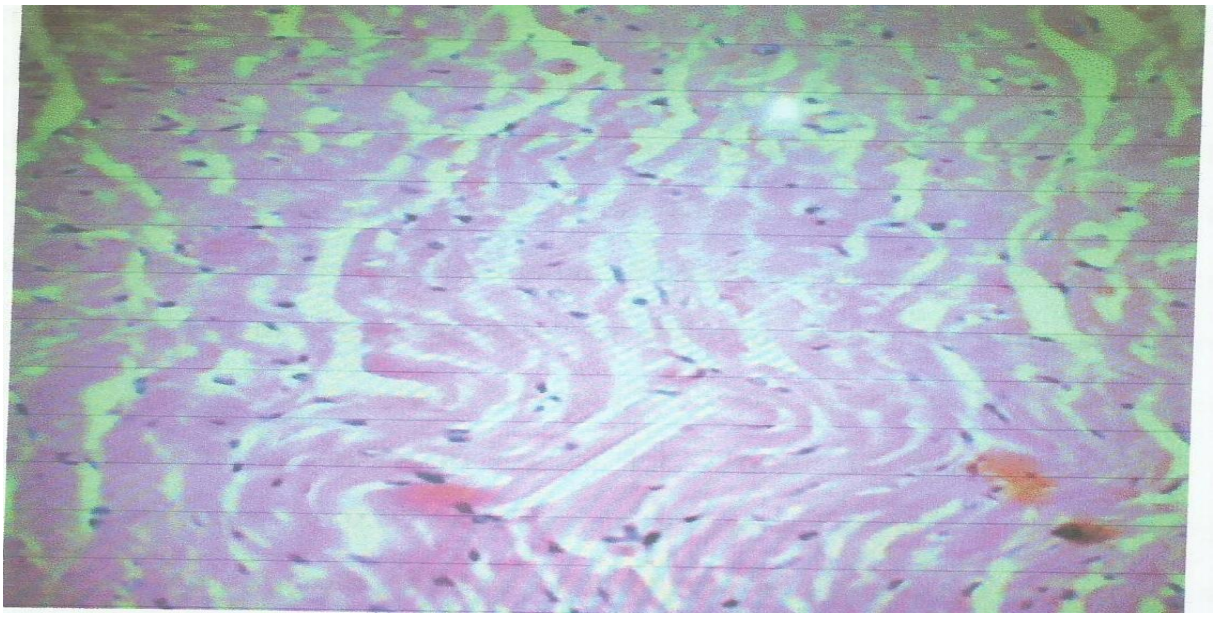

Figure 4: Microphotographing of the fourth rat heart sample. No visible cardiac damage.
Cardiac damage appears invisible during early examination in this extract treated group (group 5) from which a rat heart sample was used. Fluorescein isothiocyanate Isomer I (FITC) (19365A) and its derivatives label used, eosin used and ~1nm (0.001µm) in this tissue section.

DISCUSSION

Plants have always been a good source of drugs. Numerous studies have shown that a wide variety of plant extracts are effective in the cardiovascular system (Deeni and Sadiq, 2002). Caffeine + Paracetamol is a common pain relief drug widely used. The instant death of the rats was as a result of arrhythmias which led to collapsed veins (Finegold *et al.*, 2012). Effects observed were time and dose dependent. The aqueous extract of *Beta vulgaris* and *Viscum album* may have exhibited cardiac activity due to its antioxidant property attributable to flavonoids (Shanthi *et al.*, 2011).

The results indicate that treatment with extracts of Beetroot (*Beta vulgaris)*/Mistletoe (*Viscum album),* after establishment of Caffeine + Paracetamol-induced heart disorders significantly reduced and even reversed the disorders in rats. Hence, the extracts of *Beta vulgaris* and *Viscum album* might be effective cardiac-protectors in the diets of normal patients and patients with cardiovascular disorder since the model of caffeine + paracetamol-induced heart damage in rats simulates many of the features of human heart-lesion (disorders), although, for all essentially normal (Shanthi *et al.*, 2011). In this research work, caffeine and paracetamol were used, it was suggested that more over-the counter drugs be investigated on their cardiovascular effects in further research. Also, more research needs to be done on the cardiovascular properties and pharmacology of mistletoe plant. Effects of pure caffeine on the cardiovascular system can be investigated in future research work.

REFERENCES

Baker, F. J. and Silverton, R. E. (2005). Introduction to Medical Laboratory Techniques, 7th edition, Butterworts, London. pp 310-330.

Deeni, Y. Y. and Sadiq., N. M. (2002). Anti-microbial properties phytochemical constituents of the leaves of African Mistletoe (*Loranthacea),* An ethanomedicinal plant in Hausaland, Northern Nigeria. *Journal of ethno pharmacology.* 8393:235-240.

Finegold, J. A.; Asaria, P., and Francis, D. P. (2012). Mortality from ischaemic heart disease by country region, and age. Statistics from World Health Organization and United Nations. *International Journal of Cardiology* 168(2): 934-945.

Grubben, G. J. H. and Denton, O. A. (2004). Plant resources of tropical African vegetables. *Prota foundation wageningen;* Backhuys, Leiden; CTA.

Hobbs, D. A.; Kaffa, N.; George, T. W.; Methven, L. and Lovegrove, J. A. (2012). Blood pressure-lowering effects of beetroot juice and novel beetroot-enriched bread product in normotensive male subjects. *British Journal of nutritive* 108 (11): 2066-2074.

Ibekwe, S. E.; Uwakwe, A. A. and Monanu, M. O. (2007). *In vivo* effects of sodium benzoate on plasma aspartate aminotransferase and alkaline phosphatase of wistar albino rats. *Scientific Res. & Essay*, 2(1):010-012.

Kaplan, L. A. and Pesce, A. J. (2001). Clinical chemistry. 4th ed. St. Louis, M. O. : Mosby. p. 175.

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 43-49
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Kim, K. A.; Lee, W. K.; Kim, J. K.; Seo, M. S.; Lim, D.; Lee, K. H. *et al.*, (2001). Mechanism of refractory ceramic fibre- and rock wool-induced cytotoxicity in alveolar macrophages. Arch. Occup. Environ. Health. 74: 9-15.

Larsen, E. N. (2007). Effect of dietary nitrate on oxygen cost during exercise. Acta. Physiol. (oxf.).

Ljunberg, J. O. and Weitzberg, E. (2011). Roles of dietary inorganic nitrate in Cardiovascular health and disease. Cardiovascular. Health and disease. Cardiovasc. Res. 89(3); 525-532.

Ljunberg, J.O.; Weitzberg, E. and Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat. Rev. Drug Discov.* pp. 333-664.

Monanu, M. O. (2004). Fundamentals of Enzyme Biochemistry. pp. 166. ISBN: 978-8020-96-8

Polter, K.; Hankey, G. J.; Green, D. J.; Eikelboom, Arnolda, L. F. (2008). Homocysteine or Renal Impairment: which is the real cardiovascular risk factor? *Arteriosclerosis, Thrombosis and Vascular Biology* 28 (6):1158.

Scheck, A. C.; Perry, K.; Hank, N. C. and Clark, W. D. (2006). Anticancer activity of extracts derived from the mature roots of *Scutellaria baicalensis* on human malignant brain tumor cells. BMC Complementary and Alternative Medicine, 6, 1-9.

Shanthi, E.; Pekka, P. and Bo, N. (2011). Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization in Collaboration with the World Heart Federation and the World Stroke Organization. ISBN 978-92-4-156437-3.

Venuraju, S. M.; Yerramasu, A.; Corder, R. and Lahiri A. (2010). Osteoproteger in as a predictor of coronary artery disease and cardiovascular mortality and morbidity. *J. Am. Coll. Cardiol.* 55 (19): 2049-61.