http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (6): 882-887, 2008
ISSN 1028-8880

Effect of Grape Seed Extract on Lead Induced Hypertension and Heart Rate in Rat

Mohammad Badavi, Fatemeh Zarea Mehrgerdi, Alireza Sarkaki,
Mohammad Kazem Gharib Naseri and Mahin Dianat
Physiology Research Center, Department of Physiology, Faculty of Medicine,
Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran

Abstract: The main objective of this study was to evaluate the potential protective effect of red Grape Seed Extract (GSE) on lead induced hypertension (HTN) and Heart Rate (HR) in male Wistar rats. The rats were randomly assigned to one of 4 groups: Each group received lead acetate (100 ppm in drinking water), GSE (100 mg kg^{-1}, orally) or Lead + GSE for 45 days. Another group assigned as control group provided with tap water and regular pellet food. The Systolic Blood Pressure (SBP) and heart rate were determined by tail plethysmography coupled to a computer system. There was a sustained elevation of SBP in lead exposed rats that significantly increased at day 18 (lead treated, 112.7±2.7 mmHg, vs. control, 105.6±2.6 mmHg, n = 10, $p<0.05$) and reached a maximum level at day 36 (lead treated, 124.9±2.3 mmHg, vs. control, 103.6±3.1 mmHg, n = 10, $p<0.001$). However, the other three groups; showed no significant changes in SBP. Furthermore, the heart rate was increased sustainly in lead exposed animals that was statistically significant at days 36 and 45 (lead treated group, 404.5±9.4 vs. control group, 381.7±6.7, n = 10, $p<0.05$). The blood lead level in both lead and lead + GSE treated groups was increased significantly compared with control and GSE treated groups ($p<0.001$). However, GSE administration had no effect on the blood lead level in lead treated group. According to the result of this study, it may be concluded that GSE could have beneficial effect in protecting the cardiovascular system through its antioxidant activity against oxidative stress.

Key words: Lead, grape seed extract, rat, hypertension, oxidative stress, heart rate

INTRODUCTION

Lead is a bluish-gray metal and cumulative poison that exists in combination with organic and inorganic compounds. Chronic exposure to low levels of lead causes hypertension (HTN) in humans and animals (Gonick *et al*., 1997; Ni *et al*., 2004; Vaziri *et al*., 1997, 1999b; Vaziri and Sica, 2004). Although different considerations have been raised to explain the pathogenesis of lead-induced hypertension but the mechanism is not defined clearly. Several studies have suggested the primary involvement of the increased production of Reactive Oxygen Species (ROS) observed in lead-exposed animals (Gonick *et al*., 1997; Vaziri *et al*., 1997). Other studies revealed strong evidence for increased hydroxyl radical (OH) activity in rats with lead-induced HTN and lead-treated cultured endothelial cells (Ding *et al*., 2000, 2001). It has been previously shown that increased ROS leads to enhanced NO inactivation, depressed NO bioavailability and compensatory up regulation of NO synthases (NOSs) in rats with lead-induced HTN (Gonick *et al*., 1997; Ni *et al*., 2004; Vaziri *et al*., 1997, 1999b; Vaziri and Sica, 2004). In addition, lead induced hypertension is accompanied by a marked increase in plasma and tissue lipid peroxidation products, an increase in malodialdehyde (MDA) and substantial reduction in urinary excretion of stable NO metabolites (NOX) (Ding *et al*., 2001; Gonick *et al*., 1997; Vaziri *et al*., 1997). In many studies it has been demonstrated marked amelioration of hypertension together with normalization of plasma MDA concentration and urinary NOX excretion with a variety of antioxidants including: lazaroid (Vaziri *et al*., 1997) dimethylthiourea (Ding *et al*., 2001), vitamin E (Vaziri *et al*., 1999b) and vitamin C (Marques *et al*., 2001).

In animals exposed to lead in tap water, lead exposure affects the renin-angiotensin system, inducing sympathetic hyperactivity and increasing sensitivity to stimulation of cardiac and vascular β receptors and dopaminergic receptors (Boscolo and Carmignani, 1988; Victery, 1988). The involvements of sympathetic nervous system (SPNS) and circulating catecholamines have been implicated in lead-induced hypertension. *In vitro* electrophysiological study showed that superfusion of a low concentration (5 μM) of $PbCl_2$ enhanced excitatory postsynaptic potentials (EPSPs) in some of the SPNS examined but reduced inhibitory postsynaptic potentials (IPSPs) in other SPNS tested. On the other hand *in vivo*

Corresponding Author: Dr. Mohammad Badavi, Physiology Research Center, Department of Physiology, Faculty of Medicine, Ahwaz Jundishapour University of Medical Sciences, Ahwaz, Iran
Tel: +98-611-3332368 Fax: +98-611-3361544

study showed that intrathecal injection of $PbCl_2$ (10 and 100 nmol) increased both the heart rate and mean arterial pressure (Lai *et al*., 2002).

Grape seed is a waste product of the winery and grape juice industries. The composition and properties of grape seed have been extensively investigated and reported to have many favorable effects on human health such as lowering of Low-Density Lipoprotein (LDL), reduction of cardiovascular disease and cancer (Kim, 2005; Nomoto *et al*., 2004). In addition, the seed extract of *Vitis vinifera* (GSE) are reported to have antimicrobial and free radical scavenging properties and to be a good source of proanthocyanidins (Roychowdhury *et al*., 2001; Shi *et al*., 2003). Proanthocyanidins are potent natural antioxidants of various polyphenolic components (Shi *et al*., 2003). These compounds posses a broad spectrum of antioxidative properties with greater potency than vitamin E and C, that protects the organs against free radicals and oxidative stress, both *in vitro* and *in vivo* (Aldini *et al*., 2003; Roychowdhury *et al*., 2001). However, up to now there was no investigation carried out on the effect of GSE on the lead-induced hypertension. We hypothesized that grape seed extract could play an important role in the scavenging of free radicals and could thereby reduce the lead induced HTN. Therefore, the main objective of this study was to determine the possible changes in arterial blood pressure and heart rate in sub-chronic lead-exposed rats and to evaluate the potential protective effect of red grape seed hydro-alcoholic extract in these changes.

MATERIALS AND METHODS

Animals and treatments: The study protocols were approved by the Physiology Research Center Ethics Committee for animals and were performed according to the international conventions on animal experimentation. Experiments were carried out during April to June 2007 with the use of 40 male Wistar rats at 3 months of age, at the beginning of the experiment. The animals were housed in a climate controlled, light-regulated space with 12 h light and dark cycles. They had free access to a regular rat chow food. The rats were randomly assigned to one of 4 groups (10 rats each): Group A (control) received tap water; Group B, provided with tap water contained 100 ppm lead acetate for 45 days; Group C, provided with tap water and received GSE (100 mg kg^{-1}, orally, once a day) via gavage for 45 days. Group D, provided with tap water contained 100 ppm lead acetate and GSE (100 mg kg^{-1}, orally, once a day) via gavage for 45 days. All groups received normal chow pellet food.

Grape seed extract preparation: Grape, as large clusters with red berries, was bought from a local super market in Ahwaz, Iran and identified by botanist as *Vitis vinifera* L. Grape seeds were separated from the grapes manually, air-dried (in shade, 25-30°C) for one week and milled to fine powder (a particle size of < 0.4 mm). The grape seed powder was macerated in 70% ethanol (25% w/v) for 72 h at room temperature and was stirred three times a day. The mixture filtered with cheese cloth and the filtrate dried at room temperature (25-30°C) to remove ethanol and grape seed extract was obtained as a powder (yield: 25-30%).

Heart rate and blood pressure recording: At the beginning and day 9, 18, 27, 36 and 45 of the experiments conscious rat were placed in a restrainer, prewarmed and allowed to rest for about 20 min prior to blood pressure and heart rate measurements, that were determined by tail plethysmography coupled to a computer system (Power Lab, AD Instruments, Australia) (Gonick *et al*., 1997). Three consecutive recordings (5 min apart) were performed and the averages of recordings were calculated for each rat.

Blood lead measurement: At the end of experiments (day 45) blood samples were collected in EDTA contained tubes through cardio-puncture and the lead content of the samples was measured by graphite atomic absorption spectrometry (Carl Ziess, Germany) following digestion of the blood in a solution of nitric acid and perchloric acid (Parsons *et al*., 2001).

Statistical analyses: Results are expressed as mean±SEM. Each of the above mentioned studies were performed in a group of ten rats. Comparisons were performed by repeated measurement ANOVA followed by LSD test. The level of statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

As expected lead exposure resulted in a marked increase in arterial blood pressure. There was a sustained elevation of Systolic Blood Pressure (SBP) although it did not reach statistical significance before day 18 (Fig. 1). The SBP in lead exposed rats significantly increased at day 18 (lead treated, 112.7±2.7 mmHg, vs. control, 105.6±2.6 mmHg, n = 10, $p<0.05$, repeated measurement ANOVA followed by LSD test) and reached a maximum level at day 36 (lead treated, 124.9±2.3 mmHg, vs. control, 103.6±3.1 mmHg, n = 10, $p<0.001$, repeated measurement ANOVA followed by LSD test) and remained constant through out the experiment

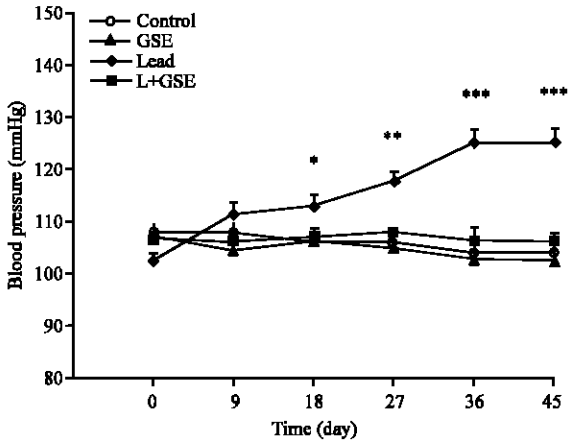

Fig. 1: Systolic blood pressure (Mean±SEM, n = 10) in different groups of rats during 45 day period of experiment. *p<0.05, **p<0.01 and ***p<0.001, significant difference between lead treated and other groups (repeated measurement ANOVA followed by LSD test)

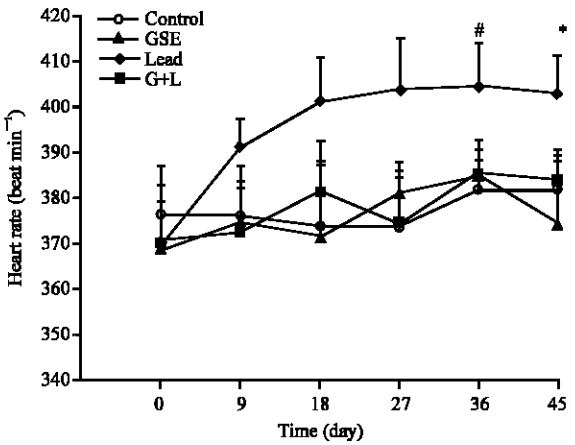

Fig. 2: Heart rate (Mean±SEM, n = 10) in different groups of rats during 45 day period of experiment. # p<0.05, lead treated group vs. control group and *p<0.05, lead treated group vs. GSE treated group (repeated measurement ANOVA followed by LSD test)

period Fig. 1. However, administration of GSE along with lead resulted in a significant prevention of the blood pressure to increase in the GSE+lead treated group. In contrast to the lead-exposed animals, the other three groups; control, GSE and lead+GSE treated animals, showed no significant changes in SBP.

There was a sustained increase in heart rate in lead exposed animals that reached its maximum at day 36 and it was statistically different from control group (lead treated group, 404.5±9.4 vs. control group, 381.7±6.7, n = 10, p<0.05, repeated measurement followed with LSD test, Fig. 2). Nevertheless, in those animals that received GSE+lead, the heart rate was not different from control or GSE treated groups and remained relatively constant.

The blood lead level in both groups, lead and lead+GSE treated groups was increased significantly compared with control and GSE treated groups (lead exposed group, 259±12 μg dL^{-1}, vs. control group, 65±8 μg dL^{-1}, n = 10, p<0.001, One way ANOVA, followed by LSD test). However, GSE administration had no effect on the blood lead level in lead treated group (Fig. 3).

The present study investigated for the first time whether GSE had antihypertensive effects on the lead-induced hypertension. In this study, we used rats, which were treated with 100 ppm lead acetate in their drinking water, for 45 days. This amount of lead is considered as

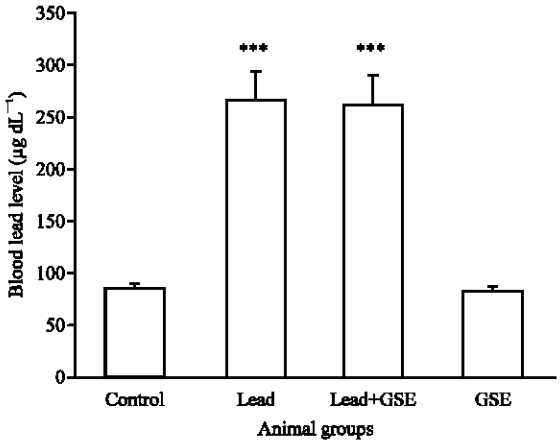

Fig. 3: Blood lead level (Mean±SEM, n = 10) in control animals and in different groups of rats after 45 days treatment with Lead (100 ppm), GSE (100 mg kg^{-1}, orally) or lead + GSE. ***p<0.001 significantly differ from control or GSE treated rats (one-way ANOVA followed by LSD test)

low level of exposure, similar to the level seen in the environment, although the result values are not directly comparable to those of humans. Previous studies have shown that exposure for longer duration (three and more months) to low level (100 ppm) of lead, not high level (5000 ppm), results in hypertension in rats (Khalil-Manesh *et al*., 1993; Purdy *et al*., 1997). Present results

show that sub-chronic (45 day) exposure will also results in increased systolic blood pressure and heart rate. These effects were prevented when the GSE was simultaneously administered with lead, but it has no effect on blood pressure and heart rate when administered alone.

Lead-induced hypertension is the element of chronic lead intoxication syndrome that has received more attention in the past decade and different considerations have been raised to explain the pathogenesis of lead-induced hypertension. Several studies have focused on the involvement of endothelium dysfunction to generate NO in this model of hypertension. These studies demonstrated the existence of an altered NO synthesis, probably because of a diminished eNOS activity and/or increased NO catabolism by oxygen free radicals. *In vitro* studies have demonstrated that NOS activity is inhibited by lead (Mittal *et al*., 1995) and previous study from Vaziri *et al.* (1999a) reported that a rise in vascular eNOS expression in lead treated rats was accompanied by inhibition of eNOS activity. In this regard, Vaziri *et al.* (2003) and Ding *et al.* (2000) have demonstrated an increased lipid peroxidation and enhanced hydroxyl radical generation in rats and cultured endothelial cells after exposure to lead, respectively. In several earlier studies, some evidence was found that oxidative stress and increased ROS activity lead to enhance NO oxidation and depressed NO bioavailability in rats with lead-induced HTN (Ding *et al*., 2001; Gonick *et al*., 1997; Vaziri *et al*., 1997, 1999a). They have further shown that lead-induced oxidative stress is primarily due to increased hydroxyl radical generation in both intact animals and cultured endothelial cells (Ding *et al*., 2000, 2001).

In another study, it has shown that lead may exert a stimulatory effect on sympathetic preganglionic neurons (SPNs). This effect may result mainly from the reduction of inhibitory postsynaptic potentials (IPSPs) and to a lesser extent, enhancement of excitatory postsynaptic potentials (EPSPs) in SPNs by low concentration of lead. The activation of SPNs may cause an enhancement of sympathetic outflow resulting in an increase of blood pressure and heart rate (Lai *et al*., 2002). In addition, it has shown that chronic exposure to lead is able to strongly increase plasma levels of adrenaline and, mostly, noradrenaline well agrees with other data showing lead to increase sympathetic nerve activity (Carmignani *et al*., 2000).

Grapes (*Vitis vinifera*) are one of the most widely consumed fruits worldwide and are rich in polyphenols. Grape seeds are byproducts formed during the industrial production of grape juice and wine. They are a potent source of proanthocyanidins, which are mainly composed of dimers, trimers and oligomers of monomeric catechins (Agarwal *et al*., 2007; Veluri *et al*., 2006). Although the mechanism of the beneficial health effects of grape seed polyphenols is not well understood, several lines of evidence strongly suggest that they are powerful antioxidants and are able to serve as free radical scavengers (Joshi *et al*., 2001; Mittal *et al*., 1995). In addition, it has shown that the polyphenols of grape seed could protect against cardiac cell apoptosis via the induction of endogenous cellular antioxidant enzymes (Du *et al*., 2007). The antioxidant activity of grape seed's polyphenols is more potent than vitamin C and vitamin E (Aldini *et al*., 2003). There are many evidences indicating that up regulation of reactive oxygen species play an important role in some forms of cardiovascular disease, including hypertension (Ademuyiwa *et al*., 2005; Patrick, 2006). Thus, GSE could prevent lead induced hypertension by scavengering ROS and/or by induction of cellular antioxidant enzymes. Furthermore, it is likely that GSE by reduction of heart rate as shown by this study could attenuate the lead induced hypertension. By this mean GSE could protect against the harmful effect of ROS on cardiovascular system through its antioxidant activity and by this mean may be have beneficial effect in protecting the organism against oxidative stress. Whether, it is protect the NO/cGMP, the endothelial cells or stimulate other relaxant system or inhibits production of vasoconstrictive substances is unclear. However, the exact mechanism by which the extract prevent lead induced hypertension remained to be determined.

ACKNOWLEDGMENTS

This research is supported by Research Affairs of Ahwaz JundiShapour University of Medical Sciences (grant No. PRC-12) and is a part of M.Sc. student thesis of Mrs. Fatemeh Zarea Mehrgerdi M.Sc. student of Physiology.

REFERENCES

Ademuyiwa, O., R.N. Ugbaja, F. Idumebor and O. Adebawo, 2005. Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria. Lipids Health Dis. [Serial online] 4: 19. http://www. lipidworld. com/content/4/1/19 Accessed January 12, 2008.

Agarwal, C., R. Veluri, M. Kaur, S.C. Chou, J.A. Thompson and R. Agarwal, 2007. Fractionation of high molecular weight tannins in grape seed extract and identification of procyanidin B2-3, 3'-di-O-gallate as a major active constituent causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. Carcinogenesis, 28 (7): 1478-1484.

Aldini, G., M. Carini, A. Piccoli, G. Rossoni and R.M. Facino, 2003. Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: New evidences for cardio-protection. Life Sci., 73 (22): 2883-2898.

Boscolo, P. and M. Carmignani, 1988. Neurohumoral blood pressure regulation in lead exposure. Environ. Health Perspect., 78: 101-106.

Carmignani, M., A.R. Volpe, P. Boscolo, N. Qiao, G.M. Di, A. Grilli and M. Felaco, 2000. Catecholamine and nitric oxide systems as targets of chronic lead exposure in inducing selective functional impairment. Life Sci., 68 (4): 401-415.

Ding, Y., H.C. Gonick and N.D. Vaziri, 2000. Lead promotes hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells. Am. J. Hypertens., 13 (5, part 1): 552-555.

Ding, Y., H.C. Gonick, N.D. Vaziri, K. Liang and L. Wei, 2001. Lead-induced hypertension. III. Increased hydroxyl radical production. Am. J. Hypertens., 14 (2): 169-173.

Du, Y., H. Guo and H. Lou, 2007. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. J. Agric. Food Chem., 55 (5): 1695-1701.

Gonick, H.C., Y. Ding, S.C. Bondy, Z. Ni and N.D. Vaziri, 1997. Lead-induced hypertension: Interplay of nitric oxide and reactive oxygen species. Hypertension, 30 (6): 1487-1492.

Joshi, S.S., C.A. Kuszynski and D. Bagchi, 2001. The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. Curr. Pharm. Biotechnol., 2 (2): 187-200.

Khalil-Manesh, F., H.C. Gonick, E.W. Weiler, B. Prins, M.A. Weber and R.E. Purdy, 1993. Lead-induced hypertension: Possible role of endothelial factors. Am. J. Hypertens., 6 (9): 723-729.

Kim, H., 2005. New nutrition, proteomics and how both can enhance studies in cancer prevention and therapy. J. Nutr., 135 (11): 2715-2718.

Lai, C.C., H.H. Lin, C.W. Chen, S.H. Chen and T.H. Chiu, 2002. Excitatory action of lead on rat sympathetic preganglionic neurons *in vitro* and *in vivo*. Life Sci., 71 (9): 1035-1045.

Marques, M., I. Millas, A. Jimenez, E. Garcia-Colis, J.A. Rodriguez-Feo, S. Velasco, A. Barrientos, S. Casado and A. Lopez-Farre, 2001. Alteration of the soluble guanylate cyclase system in the vascular wall of lead-induced hypertension in rats. J. Am. Soc. Nephrol., 12 (12): 2594-2600.

Mittal, C.K., W.B. Harrell and C.S. Mehta, 1995. Interaction of heavy metal toxicants with brain constitutive nitric oxide synthase. Mol. Cell Biochem., 149-150: 263-265.

Ni, Z., S. Hou, C.H. Barton and N.D. Vaziri, 2004. Lead exposure raises superoxide and hydrogen peroxide in human endothelial and vascular smooth muscle cells. Kidney Int., 66 (6): 2329-2336.

Nomoto, H., M. Iigo, H. Hamada, S. Kojima and H. Tsuda, 2004. Chemoprevention of colorectal cancer by grape seed proanthocyanidin is accompanied by a decrease in proliferation and increase in apoptosis. Nutr. Cancer, 49 (1): 81-88.

Patrick, L., 2006. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation and treatment. Alt. Med. Rev., 11 (1): 2-22.

Parsons, P.J., C. Geraghty and M.F. Verostek, 2001. An assessment of contemporary atomic spectroscopic techniques for the determination of lead in blood and urine matrices. Spectrochimica Acta Part B: Atomic Spectroscopy, 56 (9): 1593-1604.

Purdy, R.E., J.R. Smith, Y. Ding, F. Oveisi, N.D. Vaziri and H.C. Gonick, 1997. Lead-induced hypertension is not associated with altered vascular reactivity *in vitro*. Am. J. Hypertens., 10 (9): 997-1003.

Roychowdhury, S., G. Wolf, G. Keilhoff, D. Bagchi and T. Horn, 2001. Protection of primary glial cells by grape seed proanthocyanidin extract against nitrosative/oxidative stress. Nitric Oxide, 5 (2): 137-149.

Shi, J., J. Yu, J.E. Pohrly and Y. Kakud, 2003. Polyphenolics in grape seed biochemistry and functionality. J. Med. Food, 6 (4): 291-299.

Vaziri, N.D., Y. Ding, Z. Ni and H.C. Gonick, 1997. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: Effect of lazaroid therapy. Kidney Int., 52 (4): 1042-1046.

Vaziri, N.D., Y. Ding and Z. Ni, 1999a. Nitric oxide synthase expression in the course of lead-induced hypertension. Hypertension, 34 (4): 558-562.

Vaziri, N.D., K. Liang and Y. Ding, 1999b. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int., 56 (4): 1492-1498.

Vaziri, N.D., C.Y. Lin, F. Farmand and R.K. Sindhu, 2003. Superoxide dismutase, catalase, glutathione peroxidase and NADPH oxidase in lead-induced hypertension. Kidney Int., 63 (1): 186-194.

Vaziri, N.D. and D.A. Sica, 2004. Lead-induced hypertension: Role of oxidative stress. Curr. Hypertens. Rep., 6 (4): 314-320.

Veluri, R., R.P.S. Lingh, Z. Liu, J.A. Thompson, R. Agarwal and C. Agarwal, 2006. Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic death of DU145 human prostate carcinoma cells. Carcinogenesis, 27 (7): 1445-1453.

Victery, W., 1988. Evidence for effects of chronic lead exposure on blood pressure in experimental animals: An overview. Environ. Health Perspect., 78: 71-76.