ISSN 214-453-2

THE POSSIBLE MODULATORY EFFECTS OF KOLAVIRON ON ARSENITE-INDUCED HEPATOTOXICITY IN EXPERIMENTAL RATS

O. OLUFUNMILAYO HANNAFI AND THEODORE AKUNNA
Drug Metabolism and Toxicology Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
[hannafiazeezat@yahoo.com]

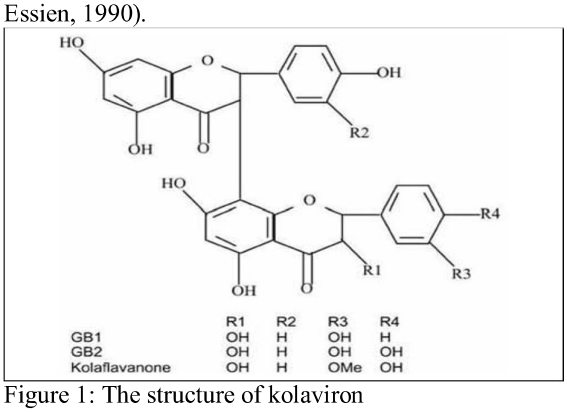
ABSTRACT
The effects of kolaviron , an active component of Garcinia kola seeds were investigated on sodium arsenite-induced hepatotoxicity in rats. Administration of a single dose of sodium arsenite (10mg/kg body weight) orally, increased the activities of serum marker enzymes-Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). This also led to increase in lipid peroxidation and reduction in the activities of antioxidant enzymes such as superoxide dismutase(SOD) and catalase(CAT). Treatment with 100mg/kg body weight of kolaviron for thirteen days before and after induction of arsenite toxicity reduced the serum activities of ALT and AST significantly ($p<0.05$). Levels of endogenous antioxidant (SOD, CAT, GST (glutathione-s-transferase) and GSH (reduced glutathione) were also elevated significantly in rats treated with kolaviron. This study revealed that kolaviron possess antioxidant and hepatoprotective property against hepatotoxicity caused by arsenite.

Keywords: *Kolaviron, Sodium Arsenite, Lipid peroxidation, Antioxidants, Hepatotoxicity.*

INTRODUCTION

Arsenic, a naturally occurring element, has posed a global health threat due to its wide distribution and adverse health effects. As an environmental agent, it is considered to be a very high priority toxic substance due to its carcinogenic potentials in humans (Bishayi B. 2000).The biochemical mechanism of arsenic toxicity lies in its binding to the sulphydryl groups of protein, resulting in the inhibition of numerous cellular enzyme systems (Sqibb et al, 1983). It is also reported that the cellular destruction of damaged thiol proteins may produce toxic oxygen radicals (Lee and Ho, 1994). It has been shown that there is a positive correlation between lipid peroxidation and arsenic tissue concentration in the livers, kidneys, and hearts of arsenite treated rats (Ramos et al, 1995).

Kolaviron, a biflavonoid, is the active component of *Garcinia kola* seeds (bitter kola). *Garcinia kola* contains a complex mixture of bioflavonoids, prenylated benzophones and xanthones (Akintonwa and Essien, 1990).



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Figure 1: The structure of kolaviron

Kolaviron is an extract of *Garcinia kola* seed which contains garcinia bioflavonoids GB1,GB2, and kolaflavonones in an approximate ratio of 2:2:1 (Terashima et al,1995, andCotterhill et al, 1978). Kolaviron has been reported to significantly prevent hepatotoxicityinduced by several hepatotoxic agents. E.g. phailloidin, thioacetamide, and paracetamol Cotterhill et al, 1978, andIwu et al, 1987). The ability of kolaviron to scavenge hydroxyl radicals by inhibiting the oxidation of deoxyribose and lipid peroxidation has been reported (Farombi et al, 2000a). Kolaviron was also reported to inhibit H_2O_2 and GSH/Fe^{3+} induced strand breaks in human lymphocytes as well as rats liver cells (Farombi et al, 2004).

Liver is the major metabolic organ of arsenic with highest concentration of arsenic retention (Benramdaneet al, 1999). The liver functions in maintaining the body metabolic homeostasis, nutrients homeostasis,filtration of particulate, protein synthesis,and also biotransformation. All these functions can be altered by liver injury resulting from acute or chronic exposure to toxicants. The general index of hepatic toxicity is the leakage of serum marker enzymes (e.g.ALT and AST) into the circulation (Tseng et al, 1991). ALT and AST are the most frequently measured indicators of hepatotoxicity. This is because they are cytoplasmic in location and are easily released into circulation after cellular damage.

This present study was designed to assess the hepatoprotective effects of kolaviron in experimental rats exposed to acute arsenite toxicity.

MATERIALS AND METHODS

Experimental animals

Thirty two wistar rats weighing 160-170g obtained from the primate colony of Biochemistry Department, University of Ibadan were used for the experiment.

Chemicals

All the chemicals used are of analytical grades.

Plant material

Garcinia kola seeds werepurchased from a local market in Ibadan, Oyo state, Nigeria..

Extraction of kolaviron

Kolaviron was extracted from the seeds of Garcinia kola seeds according to Iwu et al, 1990.The seeds were sliced, air dried, and powdered. This powder was then defatted with N-hexane for 24 hours in a soxhlet extractor. The defatted dried marc was repacked and extracted with methanol. Kolaviron was fractionated from the concentrated methanolic extract using chloroform to give a golden yellow solid which is amixture of Garcinia biflavonones-GB1, GB2, and kolaflavonones (Iwu et al1990).

Experimental design

The experimental rats were acclimatized in the Animal House of Biochemistry Department, University of Ibadan for two weeks and randomly divided into four groups of eight animalsper group as follows:

Control group: corn oil (2ml/kg body weight) for fourteen days

Arsenite group: sodium arsenite (10mg/kg body weight) was given on the first day

Pre-treatment group(KV+AS): 100mg/kg body weight of kolaviron for thirteen days

followed by sodium arsenite10mg/kg body weight on the fourteenth day.

Post-treatment group (AS+KV): sodium arsenite (10mg/kg body weight) on the first day

followed by kolaviron (100mg/kg body weight) for thirteen days.

The animals were fed and given water *ad libitum*in well-ventilated wooden cages, and maintained in a 12 hour light – dark cycle during the experimental period. At the end of the experiment, they were fasted for 24hours before they were sacrificed by cervical dislocatio

Sample preparation

The animals were sacrificed by cervical dislocation on the fifteenth day. The sera and livers were collected for biochemical analyses. The livers were quickly removed, washed in ice cold normal saline, blotted dry and weighed. Homogenization of the liver was carried out using fourvolumes of homogenizing buffer (pH 7.4) in a homogenizer. The homogenates gotten were centrifuged at 17000 x g for 20 minutes and the supernatant decanted into sample bottles and then frozen. Sera was obtained by collecting the blood into non-heparinized tube from the heart of the rats by cardiac puncture. This was allowed to stand at room temperature for about 20 minutes to clot and then centrifuged at 3500 x g for 15 minutes.

Determination of Serum Hepatospecific Enzymes

Activities of serum hepatospecific enzymes- ALT and AST were assayed using the respective Randox kits according to the method Reitman and Frankel. ALT converts L- alanine and α- ketoglutarate to pyruvate and glutamate. The pyruvate formed reacts with dinitrophenylhydrazine to produce hydrozone which in alkaline medium produces a brown complex, detectable colometrically at 546nm. On the other hand, AST catalyses the reaction between α- ketoglutarate and L-aspartate forming oxaloacetate which reacts with 2, 4 – dinitrophenylhydrazine. This in alkaline medium also gives a red brown colour, measurable at 546nm.

Estimation of Lipid Peroxidation

Lipid peroxidation in liver was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale, 1990.

Determination of reduced glutathione (GSH)

GSH was determined in the liver according to the method of Jollow et al, 1974. The method is based on the development of a relatively stable (yellow) colour when Ellman reagent is added to the sulfhydryl compound.

Determination of Glutathione-S-transferase (GST) Activity

GST activity was determined spectrophotometrically following the procedure described by Habig et al., 1974. The principle is based on the production of a complex from the enzymatic conjugation of GSH with 1- chloro-2,4-dinitrobenzene (CDNB). This has a characteristic absorption at 340nm.

Determination of Catalase (CAT) Activity

This was assessed according to the method of Sinha, 1971). The principle is based on the reduction of dichromate to chromic acetate when heated in the presence H_2O_2. Chromic acetate produced was measured colorimetrically at 570nm.

Determination of superoxide dismutase (SOD) activity

Determination of SOD activity was carried out according to the method of Misra and Fridovich,1972). The principle is based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome.

Determination of tissue proteins

Tissue protein concentrations were estimated by Biuret method using bovine serum albumin (BSA) as the standard and expressed asmg/ml BSA equivalents.

Statistical analysis

The results were reported as mean ± SD and the statistical significance of differences from control was assessed using student's t-test. A value of $p \leq 0.05$ was considered significant.

RESULT

Oral administration of sodium arsenite (10mg/kg), increased the activities of serum ALT and AST significantlywhen compared to the control group that received only corn oil (Table 1). However, after the administration of kolaviron, the enzymes significantly reduced, indicating that kolaviron (100mg/kg) was able to mitigate and repair the hepatic damage caused by sodium arsenite toxicity.

Arsenite administration did not significantly affect the activities of GST and the level of GSH ; whereas, kolaviron administration led to increases in the level of GSH and activity of GST compared to the arsenite group (Table 2).

The level of MDA (malondialdehyde) as a measure of lipid peroxidation increased significantly in arsenite group, but reduced after administering kolaviron (Table 3).

The hepatic activities of SOD and CATreducedsignificantly upon administration of arsenite (Table 4) compared with control. However, a significant increase in the activities of these enzymes was observed after kolaviron administration except for SOD in the group post treated with kolaviron.

DISCUSSION

Rats administered arsenite in this study showed significant disruption in their liver functions characterized by changes in the marker enzymes as well as by the significant reductions of the antioxidants.

Studies on animals have shown that the liver is a major target organ for arsenic toxicity because it is a vital organ for methylation of inorganic arsenite (Shen et al,2003). Fatty infiltration was observed in liver histology of male BALB/c mice exposed to 3.2mg/l arsenic in drinking water after 12 months, and fibrosis was observed after 15 months, with evidences of increasing oxidative stress in liver. There were also changes in parameters including AST, ALT, GST, GSH, MDA, and glutathione peroxidase (Santra et al, 2000).

The leakage of marker enzymes into the circulation is a general index of hepatic toxicity (Tseng et al, 1991). Serum AST and ALT activities are the most frequently measured indicators of hepatotoxicity. This is because these enzymes arecytoplasmic in location and are released into circulation after cellular damage (Sallie et al,1991). There was increase in the serum activities of AST and ALT after the induction of arsenite toxicity when compared to the control as seen in Table 1. This is most likely due to the effect of arsenite as also confirmed by the work of Leolavinothan et al., (2004)

The biflavonoid, kolaviron has severally been reported to significantly prevent hepatotoxicity induced by various hepatotoxic agents (Iwu et al, 1987, Akintonwa and Essien, 1990). Modulatory effect of kolaviron was investigated when administered both before and after arsenite toxicity. There were significant increases in the serum activities of AST and ALT of the experimental rats treated with arsenite compared to. the control group. This increase in activities of ALT and AST was reduced significantly compared to arsenite group following kolaviron administration (Table 1). No significant difference was observed between pre-treated and post-treated groups. The results obtained from this investigation indicated that kolaviron offered protection against hepatic damage precipitated by arsenite toxicity. Reduced glutathione(GSH) plays a vital role in protecting the cells from oxidative damage caused by reactive oxygen species (ROS) (Pompella et al,2003). It is a thiol peptide and antioxidant which plays an important role in many xenobiotic detoxifications. However, there was no significant difference between the GSH levels of arsenite treated group and control in this investigation (Table 2). This may be ascribed to the fact that arsenite detoxification is not only through GSH, it may also be methylated. (Waldron and Scott ,1994; IPCS 1996). There was a significant increase in GSH level of the groups sew with kolaviron compared to arsenite- treated group. This suggests the involvement of kolaviron in preservation of GSH in the system.

GST activities have been shown to be elevated in rats treated with kolaviron (Farombi, 2000a). The administration of kolaviron led to increased activities of GST (Table 2), suggesting the ability of kolaviron to induce and express drug -detoxifying enzymes. As there was no significant difference in the activities of GST between arsenite group and control, there is a possibility that arsenite can also be detoxified through methylation in the liver (IPCS 1996).

The susceptibility of membrane lipids to the deleterious action of reactive oxygen species has also been reported (Reiter, 1995). The level of MDA was very high in the group treated with arsenite compared to control group (Table 3). The lipid peroxidation observed to be induced by arsenite was significantly reversed in the groups treated with kolaviron. This reduction in lipid peroxidation after the administration of kolaviron may be due to the ability of the biflavonoid to scavenge hydroxyl radicals as reported by Farombi et al, 2000 andFarombi, 2000a.

Superoxide dismutase (SOD) and catalase (CAT) are enzymes that are very important in scavenging superoxide ion and hydrogen peroxide. They prevent production of hydroxyl radicals and protect cellular constituents from oxidative damage (Scott et al, 1991). A decrease was observed in the activities of SOD in the group treated with arsenite (Table4). The accumulation of superoxide radicals may be the reason for increased lipid peroxidation However,increase in the activities of SOD was seen in the group treated with kolaviron, maybe as a result of kolaviron's ability to scavenge the radicals generated by arsenite-induced lipid peroxidation, thus decreasing the involvementof the antioxidant enzymes in attenuating the free radicals.A similar result was also observed forcatalase (Table 4), where its activity reduced due to arsenite toxicity. The administration of kolaviron , however,led to significant recovery of catalase activity.

Table 1: the effect of kolaviron on arsenite-induced changes in ALT and AST serum activities

GROUP	ALT(IU/L)	AST(IU/L)
Control (corn oil(2ml/kg body weight))	38.40 ± 7.7	30.90 ± 2.2
Arsenite (10mg/kg body weight)	58.70 ± 3.4[a]	67.80 ± 13.0[a]
Pre-treatment	52.90 ± 6.0[ns]	39.70 ± 1.1[b]
KV(100mg/kgbody weight)+AS(10mg/kg body weight)		
Post-treatment	50.30 ± 5.3[bc]	38.3 ±7.0[b]
AS(10mg/kgbody weight)+KV(100mg/kgbody weight)		

AS: arsenite, KV: kolaviron
Values are mean ± S.D for n=8
a: significant difference when compared with the control group at p<0.05
b: significant difference when compared with the arsenite group at p<0.05
c: no significant difference when pre-treatment and post-treatment groups were compared at p<0.05
ns: no significant difference when compared with the control group. At p<0.05

Table 2: the effects of kolaviron on sodium arsenite induced changes in GSH level and GST activities in sodium arsenite rats.

GROUP	GSH(µmol/g protein)	GST ACTIVITY (µmol/min/mg protein)
Control (cornoil(2ml/kg body weight))	0.22 ± 0.04	3.78 ± 0.78
Arsenite (10mg/kg body weight)	0.20 ± 0.02[ns]	3.72 ± 0.76[ns]
Pre-treatment	0.25 ± 0.03[b]	4.77 ± 0.50[b]
KV(100mg/kg body weight)+AS(10mg/kg body weight)		
Post-treatment	0.24 ± 0.01[bc]	4.61 ± 0.57[bc]
AS(10mg/kg body weight)+KV(100mg/kg body weight)		

AS: arsenite, KV: kolaviron
Values are mean ± S.D for n=8
a: significant difference when compared with the control group at p<0.05
b: significant difference when compared with the arsenite group at p<0.05
c: no significant difference when pre-treatment and post-treatment groups were compared at p<0.05
ns: no significant difference when compared with the control group. At p<0.05.

Table 3: the effects of kolaviron on arsenite-induced lipid peroxidation

GROUP	MDA(µmol/g tissue protein)
Control (cornoil(2ml/kg body weight))	153.20 ± 19
Arsenite (10mg/kg body weight)	269.30 ± 18[a]
Pre-treatment	187.20 ± 27[b]
KV(100mg/kg body weight)+AS(10mg/kg body weight)	
Post-treatment	181.40 ± 31b[c]
AS(10mg/kg body weight)+KV(100mg/kg body weight)	

AS: arsenite, KV: kolaviron
Values are mean ± S.D for n=8
a: significant difference when compared with the control group at p<0.05
b: significant difference when compared with the arsenite group at p<0.05
c: no significant difference when pre-treatment and post-treatment groups were compared at p<0.05
ns: no significant difference when compared with the control group. At p<0.05.

Table 4: the effects of kolaviron on arsenite-induced changes in hepatic activities on SOD and CAT in experimental rats

GROUP	SOD(units/mg protein)	CAT(units/mg protein)
Control (cornoil(2ml/kg body weight))	4.85 ± 0.8	14.30 ± 0.54
Arsenite (10mg/kg body weight)	2.01 ± 0.02[a]	11.04 ± 0.81[a]
Pre-treatment	3.77 ± 0.6[b]	13.31 ± 0.92[b]
KV(100mg/kg body weight)+AS(10mg/kg body weight)		
Post-treatment	1.81 ± 0.07[ba]	12.45± 0.57[bc]
AS(10mg/kg body weight)+KV(100mg/kg body weight)		

AS: arsenite, KV: kolaviron
Values are mean ± S.D for n=8
a: significant difference when compared with the control group at p<0.05
b: significant difference when compared with the arsenite group at p<0.05
c: no significant difference when pre-treatment and post-treatment groups were compared at p<0.05
ns: no significant difference when compared with the control group. At p<0.05

CONCLUSION

Results of this investigation showed that kolaviron is effective in protecting against toxicity caused by sodium arsenite. The antioxidant mechanism is attributed to kolaviron structure(Figure 1), which possesses two flavonoid rings joined together which makes the structure unique for antioxidant and hepatoprotective function (Sreejayan and Rao 1994,Masuda et al 2001). The phytochemical analysis of kolaviron fraction has been reported to be rich flavonoids, which have been shown to have antioxidant and hepatoprotective activities (Di Carlo et al, 1999). The biflavonoid, kolaviron can therefore be said to be responsible for the observed protective effects recorded in this study.

REFERENCES

Akintonwa A., Essien A.R., 1990: Protective effects of Garcinia kola seed extract against paracetamol induced hepatotoxicity in rats. Journal of ethnopharmacology 29: 207-217

Benramdane L., Accomintti M., Fanton L., Malicier D., Vatton J.J 1999: Arsenic Speciationin human organs following fatal arsente trioxide poisoning- a case report. Clinicalchemistry. 45(2): 301-306

Bishayi B. 2000: Sodium arsenite-induced alteration in functional activity of murine peritoneal Macrophages. Indian Journal of pharmacology. 32:192-197

Cotterhill P.I., Scheinmarin F., Stenhouse T.A. 1978: Extractives from Guttiferae: kolaflavonone, a new biflavonone from the nuts of Garcinia kola heckel Journal of Chemical Society. perkin Trans 1:246

Di Carlo G., Mascolo N.,Izzo A.A., Capasso F. 1999: Flavonoids: old and new aspect of a class of natural therapeutic drugs. Life Sciences. 65(4): 337-353.

Farombi E.O., Moller P., Dragsted L.o. 2004: ex-vivo and in-vitro protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress in human lymphocytes and rats liver cells. Cell Biology and Toxicology 20:71-82

Farombi E.O., Tahnteng J.G., Agboola O., Nwakwo J.O., Emerole G.O. 2000a: Chemoprevention 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation In rats by kolaviron- a Garcinia kola seed extract. Food and Chemical Toxicology 38(6):535-541

Farombi E.O. 2000a: Mechanism for the hepatoprotective action of kolaviron: studies on Hepaticenzymes and microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. Pharmacology Research 42:75-80

Habig W.H., Pabst M.J., Jacoby W.B., 1974: Glutathione-s-transferase. The first step in mercapturic acid formation. Journal of Biochemistry 249:7130-7139.

IPCS 1996: Guide lines for drinking water quality. Health criteria and other supporting information. 2(2). Geneva . WHO.

Iwu M.M., Igboko O.A., Okunji C.O., and Tempesta M.S. 1990: Antidiabetic and aldose reductase activities of biflavanones of Garcinia kola. Journal of Pharmacy Pharmacology42(4):290-292.

Iwu M.M., Igboko O.A., Onwuchekwa U.A., Okunji C.O. 1987: Evaluation of the anti-hepatotoxicactivities of biflavonoid of Garcinia kola seed. Journal of Ethnopharmacology 21(2): 127-138.

Jollow D.J., Michelle J.R., Zampaglionic and Gillete J.R.,1974: Bromobenzene-induced liver necrosis:protective role of glutathione and evidence for 3,4-bromobenzene oxide as hepatotoxic metabolite. Pharmacology. 11:151-169.

Lee T.C., Ho I.C. 1994: Differential cytotoxic effects of arsenic on human and animal cells. Environ. Health, Perspect. 102:101-105

Leolavinothan Pari and D. Rosalin Amali. 2004: Protective role of tetra hydrocurcumin (THC)-an active principle of turmeric on chloroquine induced hepatotoxicity in rats. Journal of pharmacy and pharmaceutical science.8:115-123

Masuda T., Maekawa T., Hidaka K., Bando H., Takeda Y., Yamaguchi H., 2001: Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling Productsfrom curcuminand linoleate. Journal of Agricultural and food chemistry. 49(5):2539-2547

Misra H.P. and Fridovish I., 1972: The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The journal of biological chemistry. 247:3170-3175

Pompella A.,Visvikis A., Paolicchi A., Tata V., Casini A.F. 2003: The changing faces of glutathione-a Protagonist. Biochemical Pharmacology 66(8):1499-1503

Ramos O., Carrizales L., Yanez L.,Barriga F., 1995: Arsenic increased lipid peroxidation in rats by a mechanism independent of glutathione levels. Environmental Health Perspective. 103(1): 85- 88.

Reiter R.J., 1995: Oxidative processes and antioxidative mechanism in the aging brain. Federation of American Society for Experimental biology 9:526-53:526-53

Sallie R., Tredger J.M., William R. 1991: Drugs and the liver part 1: testing liver functions. Biopharmaceutics Drug Disposition 12: 251-259

Santra A., Maiti A., Das S., Lahiri S.,Charkaboty S.K., Guha Mazunder D.N. 2000: Hepatic damage caused by chronic arsenic toxicity in experimental animals. Clinical Toxicology38: 395-405

Scott M.D.,Lubin B.H., Zuo L., Kuypers F.A. 1991: Erythrocyte defence against Hydrogenperoxide pre eminent importance of catalase. Journal of laboratory and clinical medicine118:7-16

Shen J., Wanibuchi H., Salim E.I., Wei M., Kinoshita A., Yoshida K., Endo G., FukushimaS. 2003: Liver tumorigenicity of trimethylarsineoxide in male fischer 344 rats. Association with oxidative DNA damage and enhanced cell proliferation. Carcinogenesis. 24:1827-1835

Sinha K.A., 1971: Colometric assay of catalase. Analytical Biochemistry journal. 47:389- 94

Sqibb K.S., Fowler B.A. 1983: The toxicity of arsenic and its compounds. In: Fowler B.A, biologicalenvironmental effects of arsenic. Amsterdam: Elsevier science publishers, 233-269.

Sreejayan N., Rao M.N. 1994: Curcuminoids as potent inhibitors of lipid peroxidation. Journal of Pharmacy and pharmacology 46:1013-1016

Terashima K., Aqil M., Niwa M. 1995: A novel biflavanones from the roots of Garcinia kola. Heterocycles 41:2245-2250

Tseng J.H., Kao E.S., Chu C.Y.,Chiou F.P., Linwu H.W., Wang W.C.. 1991: Protective effects of dried Flower extract of Hibiscus sabdariffa. L. against oxidative stress in rats primary hepatocyte Food and Chemical Toxicology. 35:1159-1164

Varshney R. and Kale R.K. 1990: Effects of calmodulin antagonist on radiation induced lipid peroxidation in microsome. International Journal of Radiation Biology. 58(5):733- 743

Waldron H.A., Scott A. 1994: Metals-Arsenic, In: Raffle PAB. Adams P.H., Baxter P.j., Lee W.R. eds. Hunter's disease of occupation. 8th edition. London: Edwin Arnold. 109-112.