15

Modification by Aqueous Extracts of *Allium kurrat L.* and *Ricinus communis L.* of Cyanide Nephrotoxicity on Balb/C Mice

Fahmy G. Elsaid[1,2]
[1]*Zoology Department, Faculty of Science, Mansoura University, Mansoura*
[2]*Biology Department, Faculty of Science, King Khalid University, Abha,*
[1]*Egypt*
[2]*Saudi Arabia*

1. Introduction

1.1 Cyanide toxicity

Cyanides are components of electroplating solutions, fertilizers, fumigant mixtures, metal polishes and rodenticides. It is an environmental factor has been associated with many intoxication episodes in humans and animals resulting from the ingestion of foods, environmental pollution, chemical war, suicide, homicide, occupational factors and use in some drugs such as nitroprusside and laetrile (Watts 1998). In plants, cyanide can be found mainly as cyanogenic glycosides, as found in *Manihot* sp. (cassava), *Linum* sp., *Lotus* sp., *Phaseolus lunatu*s, *Sorghum* sp.(Conn 1978) and the content of this substance can be high as 100-800 mg/kg of the plant material (Poulton 1993). Acute poisoning ingestion by an adult of 50 ml of liquid hydrogen cyanide or 200–300 mg of one of its salts is likely to be fatal without treatment, though death is likely to be delayed for at least 1 hour. Acute cyanide toxicity is lethal, resulting in death due to respiratory failure (Greer and Jo, 1995), while chronic cyanide exposure has been implicated in the etiology of goitre (Cliff et al., 1986), tropical ataxic neuropathy (Osuntokun, 1981). Regardless the route of exposure, cyanide is rapidly absorbed into the blood stream and distributed throughout the body. Cyanide concentrates in erythrocytes through binding to methemoglobin (Towill et al., 1978) and cause hypothyroidism that leads to goiter (Kamalu and Agharanya, 1991; Elsaid and Elkomy 2006). Subacute oral administration of cyanide in rats produced changes in several biochemical indices and pathology in various organs (Tulswani et al., 2005).

1.2 Cyanide toxicity and oxidative stress

Cyanide-induced cellular oxidative stress, i.e., increase of superoxide anions, lipid peroxide, hydroxide radicals, hydrogen peroxide and others, appears to arise through multiple pathways. At the cellular level, cyanide produces chemical hypoxia by inhibiting

cytochrome c oxidase in complex IV of the mitochondrial oxidative phosphorylation chain to markedly reduce ATP (Pearce et al., 2003). As oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes under different treatment conditions. Aerobic organisms possess antioxidant defense systems that deal with ROS (Mates et al., 1999). SOD catalyzes the conversion of the highly reactive superoxide anion to O_2 and to H_2O_2 (Mates et al., 1999). This less damaging molecule can be converted spontaneously to highly reactive hydroxyl radicals. However, CAT and GSH-Px detoxify hydrogen peroxide by converting it to water before hydroxyl radicals can be produced. The propensity of cyanide to induce lipid peroxidation and impair antioxidant defense enzymes like CAT, SOD and GSH-Px are well known. Levels of MDA, GSH and GSSH are also correlated with lipid peroxidation (Ardelt et al., 1994). However, these effects were reversible after withdrawal of the poison. The oxygen radicals formed in these pathways may contribute to the oxidative damage of DNA and tumor formation. The inhibitors of CAT include azide, cyanide, GSH and dithiothreitol (Sun and Oberley 1989). GSH-Px inhibitors include cyanide and superoxide radicals (Blum and Fridovich1985). The decrease of cellular GSH following exposure to cyanide is likely due in part to reduced cellular ATP resulting from inhibition of cytochrome c oxidase (Prabhakaran et al., 2006). Antioxidants blocked the enhanced apoptosis produced by cyanide and this was directly linked to generation of ROS (Jones et al., 2003). However, levels of MDA, GSH and GSSH are also correlated with lipid peroxidation (Ardelt et al., 1994).Taken together the mechanism of hepatocyte injury resulting from inhibition of mitochondrial respiration includes a cytotoxic pathway that arises partly from an energy deficit but also from reductive stress which releases non protein bound iron from intracellular pools and induces cytotoxic ROS formation (Niknahad et al., 1995).

1.3 Antidotes and cyanide treatments

Antidotes are based on induction of methaemoglobin, which temporarily removes cyanide ions from solution. Anti-cyanide therapy may be acute, such as in poisonings, or prophylactic, as in military applications where a longer duration of action is required (Baskin and Fricke, 1992). Cyanide detoxification occurs *in vivo* mainly by conversion to thiocyanate. This reaction is catalysed by the enzyme rhodanese. In the presence of excess cyanide, the rate-limiting step is an adequate supply of sulphane sulphur (a divalent sulphur atom bonded to another sulphur atom), which can be supplied by thiosulphate (Beasley and glass 1998). However, the principal detoxification pathway of cyanide to thiocyanate in the presence of sulfur donor like garlic extract (Elsaid and Elkomy 2006) and sodium thiosulphate is mainly catalyzed by a liver mitochondrial enzyme, rhodanese (Cyanide: thiosulphate sulphur transferase) (Tylleskar et al., 1991). Cyanide accumulates in various body cells through binding to metalloproteins or enzymes such as CAT and cytochrome C oxidase. So, it is a potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the mitochondrial electron transport chain (Yen et al., 1995). The Egyptian leek contains gallic acid as a phenolic component and vitamin C, so it has antioxidant properties (Latif and ABD El-AAl, 2007). The leaves of *Ricinus communis* found to contain flavonoids like kaempferol-3-0-beta-D-rutinoside and kaempferol-3-0-beta-D-xylopyranoid and tannins (Kang et al., 1985; Khogali et al., 1992), so the present study aims to investigate the antidotal effect of *Allium* and *Ricinus* extracts.

1.4 Cyanide toxicity and renal functions

The recorded renal toxicity was also detected by the elevation in serum uric acid as well as in serum and urine creatinine and urea levels (Elsaid and Elkomy 2006). However, the protective effect of garlic on kidney indices could be attributed to its antioxidant properties because it has been found that ROS may be involved in the impairment of glomerular filtration rate (Elsaid and Elkomy 2006).

1.5 Cyanide cytotoxicity and gene expression

The rat P53 gene consists of only 10 exons. In cells with DNA injury, P53 can stop the cell cycle through p21 protein and then promote DNA repair. When DNA is seriously damaged, P53 can induce the cell to undergo programmed cell death to maintain the stability of the genome and cells. The loss of P53 function activates oncogenes and inactivates cancer suppressor genes, playing an essential role in multistage carcinogenesis (Harris 1993). Bcl-2 is constitutively expressed and localized to the outer mitochondrial membrane where it attenuates cell death signals to promote cell survival (Kroemer, 1998). Bcl-2 exerts an anti-apoptotic effect by inhibiting mitochondrial outer membrane permeabilization to suppress release of cytochrome c into the cytosol (Gross et al., 1999). Bcl-2 may also inhibit necrotic-like cell death by blocking the opening of the mitochondrial permeability transition pore to maintain cellular ATP levels within survival limits (Garland and Halestrap, 1997). Forced over-expression of Bcl-2 can block cell death produced by a variety of stimuli, including cyanide (Tsujimoto et al., 1997). Overexpression of Bcl-2 produced mitochondrial dysfunction (reduced membrane potential), caspase-independent apoptosis, and sensitization of the cells to cyanide-induced toxicity (Zhang et al., 2007). In various liver and kidney injury models, IL-4 has been shown to be both protective and deleterious. IL-4 accelerates severe hepatitis in mice deficient in suppressor of cytokine signaling proteins through activation of natural killer T cells (Naka et al., 2001), and it is believed that IL-4 plays a key role in Con A-induced hepatitis via augmentation of Vα-14 natural killer T cell-mediated cytotoxicity (Kaneko et al., 2000). The toxic effects of cyanide in kidney of rabbits have been reported after repeated exposures (Okolie and Osagie, 1999).

Although, the mechanism of toxicity of cyanide in kidney has not been delineated so far, so the study aimed to investigate the genotoxic effect of cyanide to connect the toxicity of cyanide in kidney with the physiological functions of the kidney and gene expression of some genes. Also, this work tries to discuss the antidotal effect of aqueous extracts of both *Allium kurrat* and *Ricinus communis L.,* against cyanide poisoning on kidney of Balb/c mice.

2. Material and methods

Natural Products Extracts: *Allium kurrat* (Egyptian leek), (*family Alliaceae*) and *Ricinus communis* Linn, (Castor) (*family Euphorbiaceae*) were used in this study. The leaves of leek and Castor were separately immersed in distilled water for 24 hours, filtered, stored at -20°C and freshly prepared every three days.

Animal grouping: Twenty mice weighed (45g) were used in the present experiment. The local committee approved the design of the experiments and the protocol conforms to the

guidelines of the National Institutes of Health (NIH). All measures were taken to minimize the number of mice used and their suffering. Mice were divided into four groups. Animals were caged in groups of 5, allowed standard mice chow diet and water *ad libitum* as follows: Control group; mice received no treatment. Cyanide group; mice were administered with 4.5mg/kg b.w./day of potassium cyanide (0.5 of LD_{50}) in drinking water for 30days, freshly prepared every four days for the experimental periods, with some modifications (Sousa et al., 2002). Cyanide plus *Allium kurrat* group; mice were co-administered with 4.5mg/kg b.w./day KCN in drinking water for 30 days and intraperitoneal administered with *Allium kurrat* extract at 200mg/kg b.w./day. Cyanide plus *Ricinus communis* group; mice co-administered with 9mg/kg b.w./day cyanide and intraperitoneal administered with *Ricinus* extract at 200mg/kg b.w./day for the experimental period. Animals were maintained at 22–25°C and 40–60% relative humidity with 12-h light–dark cycles. At the end of the experimental period mice were sacrificed and sera and kidney tissues were collected from each group. The kidney tissues were homogenized in a phosphate buffer solution pH 7.4, centrifuged at 4°C and the supernatant was stored at -80°C.

3. Enzymatic assay

Superoxide dismutase (SOD): This assay relied on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye (Nishikimi et al., 1972).

Catalase (CAT): It reacted with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with CAT inhibitor.

Catalase

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

In the presence of peroxidase (HRP), the remaining H_2O_2 reacted with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of CAT in the original sample, each 1unit =1µmol of H_2O_2 degraded for a minute, using a molar extinction coefficient of $43.6 M^{-1}\ cm^{-1}$ (Aebi 1984).

$$2\ H_2O_2 + DHBS + AAP \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H_2O$$

Glutathione peroxidase (GSH-Px): The activity was measured by the method described by (Ellman, 1959). Briefly, reaction mixture contained 0.2ml of 0.4M phosphate buffer pH 7.0, 0.1ml of 10mM sodium azide, 0.2ml of kidney homogenate (homogenate on 0.4M phosphate buffer, pH 7.0), and 0.2ml glutathione, 0.1 of 0.2mM H_2O_2. The content was incubated at 37°C for 10min. The reaction was arrested by 0.4ml of 10% TCA and centrifuged. Supernatant was assayed for glutathione content by using Ellman's reagent. The molar extinction coefficient for NADPH is 6220 $mM^{-1}\ cm^{-1}$ at 340nm.

Glutathione reductase (GSH-Red): GSH-Red catalyzed the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). GSH-Red is essential for the glutathione

redox cycle that maintains adequate levels of reduced cellular GSH. According to the method of Goldberg and Spooner (1983), GSH-Red catalyzed the reduction of the oxidized glutathione (GSSG) in the presence of NADPH that oxidized into $NADPH^+$. The decrease in absorbance was measured at 340nm.

$$NADPH + H^+ + GSSG \xrightarrow{\text{GSH-Red}} NADP^+ + 2GSH$$

GSH (GSH) content: GSH served as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the GSH-Px and glutathione-s-transferase in the detoxification of organic peroxide and metabolism of xenobiotics, respectively. Homogenize the tissue in 5–10ml cold buffer (50mM potassium phosphate, pH 7.5, 1mM EDTA) per gram tissue. Centrifuge at 100,000 x g for 15min at 4°C. Remove the supernatant for assay and store on ice and then freeze the sample at - 80°C. The method based on the reduction of 5,5` dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405nm (Beutler et al., 1963).

Total antioxidant capacity: The determination of the total antioxidant capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2).The antioxidants in the sample eliminated a certain amount of the provided hydrogen peroxide. The residual H_2O_2 was determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro–2–hydroxyl benzensulphonate to a colored product (Koracevic et al., 2001).

Lipid peroxidation: The thiobarbituric acid reactive substances (TBARS) as malondialdehyde were estimated by the method of Ohkawa et al., (1979). Briefly, to 0.2ml of kidney homogenate, 0.2ml of 40% sodium dodecyle sulphate, 1.5ml of 20% acetic acid (prepared in 0.27M of HCl) and 1.5ml of 0.5% thiobarbituric acid were added together. The mixture was heated for 60min at 95°C in a temperature controlled water bath to give a pink color. The mixture was then centrifuged at 3500 r.p.m. for 10min. Finally, the absorbance of the supernatant layer was read spectrophotometrically at 532nm, the molar extinction coefficient factor equal 1.56 $x10^5 M^{-1}cm^{-1}$.

Total lipid: According to the method of Zollner and Kirsch (1962), lipids reacted with sulfuric, phosphoric acids and vaniline to form pink colored complex, the absorbance was measured at 545nm.

Total protein: In the presence of an alkaline cupric sulfate, the protein produced a violet color, the intensity of which is proportional to their concentration (Gornall and Bardawill, 1949). The absorbance was read at 550nm.

Uric acid assay: According to the method of Fossati et al., (1980) by using of 3, 5-dichloro2-hydroxybenzene sulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum.

Creatinine assay: Serum creatinine level was estimated according to the method of Henry (1974).

Extraction of total RNA from kidney tissue homogenates: Total RNA was isolated from kidney homogenate using RNeasy Mini Kit according to manufacturer's instructions (QIAGEN, Germany). About 100 µl of each homogenate was subjected to RNA extraction and the resultant RNA was dissolved in DEPC-treated water, quantified spectrophotometrically and analyzed on 1.2% agarose gel. RNAs inhibitors were added to the samples during the RNA extraction process (Shati et al., 2011).

4. Real time PCR and gene expression

a- For P53 and Bcl2 genes: The extracted RNA from kidney tissues of different groups was subjected to examine the expression level of two genes using specific primers in the presence of Glyceraldehyde-3-phosphate dehydrogenase as a housekeeping gene. The Real time reaction consists of 12.5µl of 2X Quantitech SYBR® Green RT Mix (Fermentaz, USA), 2µl of the extracted RNA (50ng/ µl), 1µl of 25 pM/µl forward (F) primer, 1µl of 25 pM/µl reverse (R) primer (Table 1), 9.5 µl of RNAase free water for a total of 25 µl. Samples were spun before loading in the rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95°C for 10 min.; 40 cycles of 95°C for 15 sec, annealing at 64°C for 30 sec and extension at 63°C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene 6000 system (QIAGEN, USA).

Primer name	Primer sequence from 5`-`3	Annealing temp.
P53	F- AGGGATACTATTCAGCCCGAGGTG R-ACTGCCACTCCTTGCCCCATTC	64°C
Bcl-2	F-ATGTGTGTGGAGAGCGTCAACC R-TGAGCAGAGTCTTCAGAGACAGCC	63°C
IL-4	F-CTATTAATGGGTCTCACCTCCCAACT R-CATAATCGTCTTTAGCCTTTCCAAG	60°C
IL-12	F-CAGCCTTGCAGAAAAGAGAGC R-CCAGTAAGGCCAGGCAACAT	65°C
GPDH (House keeping gene)	F-ATTGACCACTACCTGGGCAA R-GAGATACACTTCAACACTTTGACCT	60 °C

GPDH: glyceraldehydes 6-phosphate dehydrogenase.

Table 1. Oligonucleotide primer sequences used in this study:

b- For interleukins (IL-4 and IL-12) genes: Real time PCR was performed using specific primers for two of cytokines genes, IL-4 and IL-12. The reaction was performed on the total RNA extracted from the kidney of all experimental groups. The PCR reaction constituents and conditions were similar to the above genes except that the annealing temperature was 60°C for IL-4 and 65°C for IL-12.

5. Molecular data analysis

Comparative quantitation analysis was performed using Rotor-Gene-6000 Series Software based on the following equation:

$$\text{Ratio target gene expression} = \frac{\text{Fold change in target gene expresion } \left(\frac{sample}{control}\right)}{\text{Fold change in reference gene expresion } \left(\frac{\text{house keeping gene}}{\text{control}}\right)}$$

Real-time PCR data of all samples were analyzed with appropriate bioinformatics and statistical program for the estimation of the relative expression of genes using real-time PCR and the result normalized to its gene (Reference gene). The data were statistically evaluated, interpreted and analyzed using Rotor-Gene-6000 version 1.7.

6. Statistical analysis of biochemical data

The biochemical data recorded were expressed as mean±SD and statistical and correlation analyses were undertaken using the One-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. A P value < 0.05 was statistically significant. A Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

6.1 Results

Table (2): ANOVA test showed high significant (P<0.001) changes between the different groups. The results showed very high significant decrease (P<0.001) in kidney total antioxidant capacity, decrease in the activities of CAT, SOD, GSH-Px, GSH-Red and a decrease in GSH content in cyanide group when compared with the control one. Also there was a very high significant increase (P<0.001) in TBARS in cyanide group. Cyanide & *Allium* and cyanide & *Ricinus* groups were improving the nephrotoxicity of cyanide when compared with cyanide group.

Groups Parameters	Control	Cyanide	Cyanide& *Allium*	Cyanide& *Ricinus*
T. Antioxidant capacity	0.58±.07	0.37±0.04***	0.47±0.09^{*b}	0.48±0.03^{*b}
CAT	7.7±0.58	5.4±0.83***	5.8±0.58***	6.7±0.48^{*b}
SOD	55.3±3.9	41.7±5.7***	44.6±2.6***	50.9±3.8^{b}
GSH-Px	7.0±0.42	4.8±0.58***	5.0±1.1***	5.77±0.85*
GSH-Red	59.1±3.6	42.7±3.2***	47.9±6.4***	52.2±3.4^{*b}
GSH	9.3±1.8	5.4±0.76***	6.0±0.38***	7.4±0.51^{**b}
TBARS	108.0±9.3	254.6±18.5***	162.1±33.5^{***c}	146.4±18.4^{***c}

In comparison control with other groups, p<0.05 *, p<0.01**, p<0.001***

In comparison cyanide group with cyanide& *Allium* and Cyanide& *Ricinus* groups, p<0.05 ^{a}, p<0.01 ^{b}, p<0.001 ^{c}

Table 2. Kidney antioxidants and oxidative stress in different Balb/C groups:

In table (3): ANOVA test showed high significant (P<0.001) changes between the different groups. serum creatinine and uric acid levels showed very high significant increase (P<0.001) in cyanide group in comparison with control group. From the results in cyanide& *Allium* and cyanide& *Ricinus* groups, the aqueous extracts of *Allium* and *Ricinus* showed

good effect on renal function when compared with cyanide group. The ameliorative effect appeared in decreasing of serum creatinine and uric acid levels in cyanide& *Allium* and cyanide& *Ricinus* groups. Moreover the level of total lipids and total protein content were decreased in cyanide group when compared with the control one. The treated groups with *Allium* and *Ricinus* extracts showed a good effect in managing this decrease.

Groups Parameters	Control	Cyanide	Cyanide& *Allium*	Cyanide& *Ricinus*
Serum Creatinine	0.68±0.07	1.1±0.11***	0.84±0.05**c	0.72±0.09c
Serum Uric Acid	2.0±0.19	3.3±0.52***	2.8±0.25**a	2.3±0.0.23c
Serum T. Lipids	0.72±0.05	0.42±0.05***	0.47±0.05***	0.58±0.05***c
Kidney T. Lipids	44.1±5.4	30.0±3.01***	30.8±2.81***	39.1±4.87b
Kidney T. Protein	10.5±1.4	5.6±1.1***	6.9±0.90***	7.59±1.27***a

In comparison control with other groups, $p<0.05$ *, $p<0.01$**, $p<0.001$***

In comparison cyanide group with cyanide& *Allium* and Cyanide& *Ricinus* groups, $p<0.05$ a, $p<0.01$ b, $p<0.001$ c

Table 3. Renal biomarkers in different Balb/C groups:

6.2 Molecular data

As in figure (1), P53 expression was high in cyanide group when compared with control group as shown in figure (1). But the P53 gene expression was so low in cyanide& *Allium* and cyanide& *Ricinus* groups when compared with cyanide group. Moreover, the Bcl-2 gene was highly expressed in cyanide administered rats in comparison with control group. The treated rats with cyanide& *Allium* and cyanide& *Ricinus* extracts showed amelioration expression of this gene in kidney tissue as in fig (1).

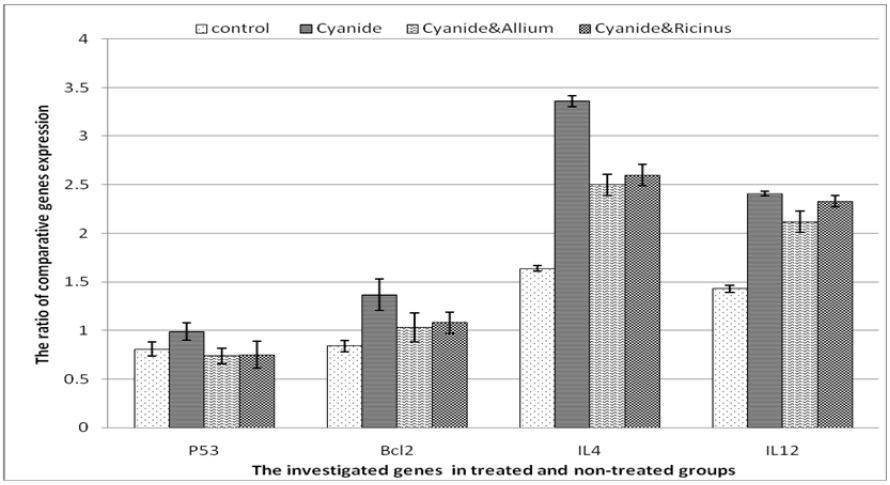


Fig. 1. The ratio of comparative gene expression in kidney of different treated groups compared with the control ones of balb/C mice, P53; Bcl-2 (B cell lymphoma 2); IL4 and IL12 (interleukin).

Also in figure (1), the expression of IL-4 and IL-12 genes was high in cyanide group in comparison with control group. But in the contrary a low gene expression was observed in cyanide& *Allium* and cyanide& *Ricinus* groups when compared with control group.

7. Discussion

7.1 Cyanide intoxication and antioxidants/oxidants balance

It was observed in KCN-treated cells that ROS generated from sources other than mitochondria represented a small portion of the total cellular ROS (Hariharakrishnan et al., 2009). Also it was indicating that mitochondria may be the primary source of ROS following cyanide (Chen et al., 2003). It is well characterized that cyanide inhibits cytochrome oxidase, which in turn stimulates ROS formation at complexes I and III. Free radicals are chemical entities that exist separately with one or more unpaired electrons. Free radicals induce damage or death of that cell of which the affected molecule is a part. The propagation of free radical formation could continue for thousands of reactions and hence the damage caused will be extensive (Campbell and Abdulla, 1995). Lipids, proteins and DNA are all susceptible to attack by free radicals. Cyanide toxicity also caused by increased generation of superoxide anion and lipid peroxidation (Daya et al., 2002) with inhibition of antioxidants enzymes (Ardelt et al., 1994). Hariharakrishnan et al., (2009) showed marked increase in total ROS and reactive nitrogen species levels and elevated lipid peroxidation after treatment with 1.25mM KCN. Reduced GSH levels render the cell more susceptible to endogenous oxidants (Slater et al., 1995).In the present study, cyanide intoxication showed increased MDA level (a marker of lipid peroxidation) with decreased CAT and SOD activities in kidney, indicating renal toxicity. In addition, the cyanide-induced oxidative stress leads to a decrease in GSH content, GSH-Red and GST activities. It exerts its effect by enhancing the non-enzymatic antioxidant as GSH and the detoxifying enzyme as GST (Saravanan et al., 2004). So it restores glutathione level and increases the activities of glutathione reductase and glutathione-S-transferase (Saravanan et al., 2004). Oxidative stress in the cells or tissues refers to enhanced generation of reactive oxygen species and/or depletion in antioxidant defense system. ROS generated in the tissues are efficiently scavenged by enzymatic antioxidant system such as GSH-Px and glutathione reductase as well as non enzymatic antioxidants such as glutathione, vitamin A, C, and E (Schlorff *et al.,* 1999). GST is a detoxification enzyme which catalyzes the conjugation of many electrophilic agents with GSH (Hayes et al., 1995) so it may be bind to CN and this explain their decrease in kidney tissues. Cyanide is known to induce oxidative stress by depleting the cellular thiol (Gunasekar et al., 1996). Similarly, in the present study, GSH, an intracellular antioxidant, was depleted after exposure to 4.5mg KCN making the cell environment less reducing and vulnerable to oxidative stress. So the changes in kidney may be induced by cyanide due to the imbalance in the antioxidant enzymes and oxidative stress as showed by (Elsaid and Elkomy 2006). In the present study, cyanide induced nephrotoxicity was reflected by the observed increases in serum uric acid and creatinine levels as well as total protein and lipid profile content (Elsaid and Elkomy 2006) as in table (3). Organosulphurs enhance the synthesis of the cellular GSH content in red blood cells (Wu et al., 2001), which is catalyzed by antioxidant enzymes (Godwin et al., 1992) also it may be alleviating the renal functions. The protective efficacy of *Allium* (Latif and ABD El-AAl, 2007) and *Ricinus* (Khogali et al., 1992) against cyanide-induced cytotoxicity can be attributed to the antioxidant property of both (Khogali et al., 1992). Also it can be interpreted by the sulphur donor properities of *Allium* (Latif and ABD El-AAl, 2007).

Moreover, cyanide is regarded as a selective neurotoxin (Pettersen and Cohen, 1993). It rapidly induces a non-competitive inhibition of the mitochondrial cytochrome c oxidase activity, which results in compromised aerobic oxidative metabolism and phosphorylation, cellular hypoxia, and lactic acidosis (Baud, 2007). Other complications of cyanide toxicity include loss of ionic homeostasis, excitotoxicity along with free radical damage (Johnson et al., 1987a). Cyanide-impaired mitochondrial energy metabolism is accompanied by severe lactic acidosis, elevated intracellular Ca^{2+} levels, decreased cellular ATP content and lipid peroxidation, leading to activation of proteases, lipases and xanthine oxidases, culminating in cell death (Bondy and Komulainen, 1988). This is may be a mechanism explains the decrease of total lipid and total protein in the kidney in cyanide group.

7.2 Cyanide intoxication and gene expression

Oxidative species such as hydrogen peroxide, superoxide, or free radical intermediates play a crucial role upstream of apoptosis activation. Oxidative stress is a key factor in cyanide-mediated apoptosis, partly by activation of redox-sensitive transcription factor $NF\text{-}_kB$ (Shou et al., 2002). Physiological effects of cyanide may be mediated through changes in the expression of gene products, either at the mRNA or protein level. Cyanide leads to stimulation of P53 expression in Cyanide group compared with the control ones. *Ricinus* and *kurrat* handle the expression of the P53 in cyanide administered mice and diminished the stimulation effect of cyanide of the P53 level in kidney cells. Activation of P53 in response to an obstruction of mitochondrial electron transport chain may additionally contribute to tissue damage (Khutornenko et al., 2010). It is worth noting that in cellular models of cyanide group, P53 was found to be conformational altered, making these cells less vulnerable to environmental stressors or genotoxic insults. But the expression of P53 was higher in cyanide& *Allium* and cyanide& *Allium* groups when compared with control one. Thus, P53 seems to play a pivotal role in cyanide detoxification, implying that modulation of cell death pathways might be of therapeutic benefit in cyanide detoxification. Apoptosis can be triggered by a variety of stimuli, including receptor ligation, growth factor withdrawal, exposure to chemotoxins, or even physical damage. Depending on the specific situation and the particular cell type, a variety of different internal signaling pathways can be initiated. However, these converge at certain control points into the execution phase of the program, where inhibitory or stimulatory cofactors like the family of Bcl-2 proteins come into action. Some members such as Bcl-2 or Bcl-xL block apoptosis, whereas others such as bax or bak promote apoptosis. Bcl-2 is apoptosis regulating gene which are functionally contradictory. The primary action of cyanide is inhibition of cytochrome c oxidase in complex IV of the oxidative phosphorylation chain, thereby blocking intracellular oxygen utilization (histotoxic hypoxia) and reducing cellular ATP generation (Leavesley et al., 2008). As a potent regulator of the Bcl-2, ROS regulate the expression of Bcl-2 via both transcription and protein degradation (Hildeman et al., 2003; Li et al., 2004). ROS-activated transcription factors such as $NF\text{-}_k$ B often negatively regulate transcription of the Bcl-2 gene (Pugazhenthi et al., 2003). Bcl-2 expression was induced in the cyanide group compared with the control one. But when cyanide group treated with aqueous *Allium* and *Ricinus* extracts low expression of Bcl-2 was observed as a result, *Allium* and *Ricinus* extracts managed the expression of the Bcl-2 by which the cell may not directed to apoptosis. Taken together, over-expression of Bcl-2 protected against the cyanide toxicity, thus providing strong evidence that Bcl-2 down-regulation contributes to the cell death (Zhang et al., 2009). Here

the data suggests that *Allium* and *Ricinus* induced P53 and suppressed Bcl-2 in the cyanide& *Allium* and cyanide& *Ricinus* groups which may help in attenuating of cyanide toxicity in the kidney cells.

Currently, it is generally believed that the role of IL-12 is a heterodimeric cytokine produced by activated blood monocytes, macrophages and glial cells. It enhances differentiation and proliferation of T cells and increases production of pro-inflammatory cytokines (Jifen et al., 2003). Cyanide makes induction for the IL-4 and IL-12 in cyanide group. But a low amount of IL-4 and IL-12 in cyanide administered rats treated with *Allium* and *Ricinus* extracts was observed as gene expression compared with control one. This confirms that both *Alluim* and *Ricinus* extract help in increasing the cyanide administered rat's immune system through modulating the interleukins expression as anti-inflammatory. To move in the end we suggest that both *Allium* and *Ricinus* play a vital role as antidotes and they are not protective but administrative.

8. Conclusion

The toxicity of cyanide associated with a decrease in the antioxidants enzymes as catalase, superoxide dismutase, glutathione and their relative enzymes and increase in the lipid peroxidation as thiobarbituric acid reactive substance in the kidney of Balb/c mice. P53 gene showed high expression in responding to cyanide toxicity as well as Bcl-2 and interleukins 4& 12 genes that showed over-expression in the kidney of mice. The watery extract of *Allium* and *Ricinus* was capable to manage the biochemical and gene expression accomplished with cyanide intoxication. So, the *Allium* and *Ricinus* have antiodotal effect at least in Balb/C mice at least in kidney tissue.

9. References

Aebi, H. (1974). Catalase. In. Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, pp. 673-677.

Ardelt, B.K., Borowitz, J.L. and Isom, G.E. (1989). Brain lipid peroxidation antioxidant protectant mechanisms following acute cyanide intoxication. Toxicology 56, 147–154.

Ardelt, B.K., Borowitz, J.L., Maduh, E.U., Swain, S.L. and Isom, G.E. (1994): Cyanide-induced lipid peroxidation in different organs: subcellular distribution and hydroperoxide generation in neuronal cells, Toxicology 89, 127–137.

Baskin, S.I., and Fricke, R.F. (1992). The pharmacology of *p*-aminopropiophenone in the detoxification of cyanide. Cardiovasc. Drug Rev. 10, 358.

Baud FJ. (2007). Cyanide: critical issues in diagnosis and treatment. Hum Exp Toxicol, 26:191–201.

Beasley, D. M. G. and Glass, W. I. (1998). Cyanide Poisoning: Pathophysiology and Treatment Recommendations. *Occup Med (Lond)*. 48: 427–431.

Beutler, E., Duron, O. and Kelly, B.M. (1963).Improved method for the determination of blood glutathione. *J Lab Clin Med* 61:882–888,

Blum, J., and Fridovich, I. (1985). Inactivation of glutathione peroxidase by superoxide radical. *Arch. Biochem. Biophys.* 240:500-508.

Bondy SC, Komulainen H. (1988). Intracellular calcium as an index of neurotoxic damage. Toxicology, 35:35–41.

Campbell, I.C. and Abdulla, E.M. (1995). Strategic approaches to in vitro neurotoxicology. In: Chang, L.W., Slikker Jr., W.J. (Eds.), Approaches and Methods. Neurotoxicology Academic Press, London, pp. 495– 505.

Chen, Q., Vazquez, E. J., Moghaddas, S., Hoppel, C. L. and Lesnefsky, E. J. (2003). Production of reactive oxygen species by mitochondria: central role of complex III. J. Biol. Chem. 278:36027–36031.

Cliff J., Lundquist P., Rosling H., Sorbo B. and Wide L (1986) Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. Acta Endocrino-logica 113, 523±528.

Cliff, J., Lundquist, P. Rosling, H. Sorbo, B. and Wide, L. (1986). Thyroid function in cassava-eating population affected by epidemic spatic paraparesis. Acta Endocrinol., 113: 523-528.

Cliff, J., P. Lundquist, et al. (1986).Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. European Journal of Endocrinology 113(4): 523.

Conn, E.E. (1978). Cyanogenesis, the production of cyanide, by plants. In. Keeler, R.F., Van Kampen, K.R., James, L.F. (Eds.). Effects of Poisons Plants on Livestock. Academic Press, San Diego, pp: 301-310.

Daya, S., Walker R.B. and kumar-Dukie, S. (2002). Cyanide-induced free radical production in rat brain homogenate is reduced by asprin. Metabolic Brain disease 15: 203-210.

Ellman, G. L. (1959).Tissue sulfhydryl groups. Archives of biochemistry and biophysics 82(1): 70-77.

Elsaid, F. G. and M. M. Elkomy (2006). Aqueous Garlic Extract and Sodium Thiosulphate as Antidotes for Cyanide Intoxication in Albino Rats. Research Journal of Medicine and Medical Sciences 1(2): 50-56.

Fossati, P. Pregncipe, L. and Berti, G. (1980.) Use of 3,5-dichloro2-hydroxybenzenesulfonic acid/4 – amino-phenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

Garland, J.M. and Halestrap, A. (1997). Energy metabolism during apoptosis. Bcl-2 promotes survival in hematopoietic cells induced to apoptose by growth factor withdrawal by stabilizing a form of metabolic arrest. J. Biol. Chem. 272, 4680–4688.

Godwin, A.K., Meister, A. O'Dwyer, P.J. Huang, C.S., Hamilton, T.C., and Anderson, M.E. (1992). High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis, Proc. Natl. Acad. Sci. U.S.A., 89: 3070-3074.

Godwin, A.K., Meister, A., O'Dwyer, P.J., Huang, C.S., Hamilton, T.C., and Anderson, M.E. (1992). High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis, Proc. Natl. Acad. Sci. U.S.A., 89: 3070-3074.

Goldberg, D.M. and Spooner, R.J. (1983). In Methods of Enzymatic Analysis (Bergmeyen, H.V. Ed.) 3rd ed. Vol. 3, pp 258-265, Verlog Chemie, Deerfield beach, FI .

Gornall, A. G. and Bardawill, C. J. (1949). "Determination of serum proteins by means of the biuret reaction." J. biol. Chem 177, (2): 751-766.

Greer, J. J. and Jo, E. (1995). Effects of cyanide on neural mechanism controlling breathing in neonatal rat in vivo. Neurotoxicology 16, 211-215.

Greer, J. J. and Carter, J. E. (1995). "Effects of cyanide on the neural mechanisms controlling breathing in the neonatal rat in vitro." Neurotoxicology 16(2): 211.

Gross, A., McDonnell, J.M. and Korsmeyer, S.J., (1999). BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 13, 1899–1911.

Gunasekar, P.G., Sun, P., kanthasamy, A.G., Borowitz, J.L. and Isom, G.E. (1996). Cyanideinduced neurotoxicity involves nitric oxide and reactive oxygen species generation after N-methyl-d-aspartate receptor activation. J. Pharmacol. Exp. Ther. 277, 150–155.

Hariharakrishnan, J., Satpute, R., Prasad, G.B.K.S. and Bhattacharya, R. (2009). "Oxidative stress mediated cytotoxicity of cyanide in LLC-MK2 cells and its attenuation by alpha-ketoglutarate and N-acetyl cysteine." Toxicology letters 185(2): 132-141.

Harris, C.C. (1993). P53: at the crossroads of molecular carcinogenesis and risk assessment, Science 262, 1980–1981.

Hayes, J.D. and Pulford, D.J. (1995). The glutathione S-transferase supergene family. Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol., 30: 445-600.

Henry, R.J. (1974). Method of creatinine. Clinical Chemistry, principles and techniques 2nd Edn., Harper and row, pp: 525.

Hildeman, D.A., Mitchell, T., Aronow, B., Wojciechowski, S., Kappler, J. and Marrack, P. (2003). Control of Bcl-2 expression by reactive oxygen species. *Proc Natl Acad Sci U S A* 100:15035–15040.

Jones, D. C., Prabhakaran, K., Li, L., Gunasekar, P.G., Shou, Y., Borowitz, J.L. and Isom G.E. (2003). Cyanide Enhancement of Dopamine-Induced Apoptosis in Mesencephalic Cells Involves Mitochondrial Dysfunction and Oxidative Stress. NeuroToxicology 24, 333–342.

Johnson JD, William GC, Kevin D, Burris. Isom GE. (1987a). Peroxidation of brain lipids following cyanide intoxication in mice. Toxicology, 46:21–8.

Kamalu, B.P. and Agharanya, J.C. (1991). The effect of a nutritionally-balanced cassava (Manihot esculenta Crantz) diet on endocrine function using the dog as a model. 2-Thyroid. Br. J. Nutr., 65: 373-379.

Kaneko, Y., Harada, M., Kawano, T., Yamashita, M., Shibata, Y., Gejyo, F. , Nakayama, T. and Taniguchi. M. (2000). Augmentation of Vα14 NKT cell-mediated cytotoxicity by interleukin 4 in an autocrine mechanism resulting in the development of concanavalin A-induced hepatitis. *J. Exp. Med. 191:105.*

Kang, S.S., Cordell, A., Soejarto, D.D., Fong, H.H.S. (1985). Alkaloids and flavanoids from *Ricinus commmunis* .J Nat Prod 48: 155-156.

Khogali, A., Barakat, S., Abou-Zeid, H. (1992). Isolation and identification of the phenolics from *Ricinus communis* L . *Delta J Sci* 16: 198-211.

Khutornenko, A.A., Roudko, V.V., Chernyak, B.V., Vartapetian, A.B., Chumakov, P.M., Evstafieva , A.G. (2010). Pyrimidine biosynthesis links mitochondrial respiration to the P53 pathway. Proc Natl Acad Sci U S A. 107 (29):12828-33.

Koracevic, D., G. Koracevic, et al. (2001). "Method for the measurement of antioxidant activity in human fluids." Journal of clinical pathology 54(5): 356.

Kroemer, G. (1998). The mitochondrion as an integrator/coordinator of cell death pathways. Cell Death Differ. 5, 547.

Latif, S. S. and ABD El-AAL, H. A. (2007). Minerals profile-shlef extension and nutritive value of fresh green leafy vegetables consumed in Egypt. Afri Crop Sci conf Proceedings, 8, 1817-1826.

Leavesley, H.B., Li, L., Prabhakaran, K., Borowitz, J.L., and Isom, G.E. (2008). Interaction of cyanide and nitric oxide with cytochrome c oxidase: implications for acute cyanide toxicity. *Toxicol Sci* 101:101–111.

Li, D., Ueta, E., Kimura, T., Yamamoto, T. and Osaki, T. (2004). Reactive oxygen species (ROS) control the expression of Bcl-2 family proteins by regulating their phosphorylation and ubiquitination. *Cancer Sci* 95:644–650.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mates, J.M., Perez-Gomez, C. and Nunez de Castro, I. (1999). Antioxidant enzymes and human diseases. Clin. Biochem. 32, 595–603.

Naka, T., H. Tsutsui, M. Fujimoto, Y. Kawazoe, H. Kohzaki, Y. Morita, R. Nakagawa, M. Narazaki, K. Adachi, T. Yoshimoto, et al., (2001). SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated crosstalk inhibition of IFN and IL-4 signaling in vivo. *Immunity 14:535*.

Niknahad, H., Khan, Peter, S. and O'Brien, J. (1995). Hepatocyte injury resulting from the inhibition of mitochondrial respiration at low oxygen concentrations involves reductive stress and oxygen activation. Chemico-Biological Interactions 98, 27-44.

Nishikimi, M., Appaji Rao, N. et al. (1972). "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen." Biochemical and Biophysical Research Communications 46(2): 849-854.

Ohkawa, H., N. Ohishi, et al. (1979). "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction." Analytical biochemistry 95(2): 351-358.

Okolie, N.P. and Osagie, A.U. (1999). Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem. Toxicol. 37, 745–750.

Osuntokun B. O. (1981). Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. World Review of Nutrition and Dietetics 36, 141-173.

Pettersen JC, Cohen SD. (1993). The effects of cyanide on brain mitochondrial cytochrome oxidase and respiratory activities. J Appl Toxicol, 13: 9–14.

Pearce, L. L., Bominaar, E. L., Hill, B. C. and Peterson, J. (2003). Reversal of cyanide inhibition of cytochrome c oxidase by the auxiliary substrate nitric oxide: an endogenous antidote to cyanide poisoning. J. Biol. Chem. 278: 52135–52145.

Poulton, J.E. (1983). Cyanogenic compounds in plants and their toxic effects. In. Keeler, R.F., Tu, A.T. (Eds.), Handbook of Natural Toxins. Marcel Dekker, New York, pp; 117–157.

Prabhakaran, K., Li, L., Borowitz, J.L. and Isom, G.E. (2006). Inducible nitric oxide synthase upregulation and mitochondrial glutathione depletion mediate cyanide-induced necrosis in mesencephalic cells. J. Neurosci. Res. 84, 1003–1011.

Pugazhenthi, S., A. Nesterova, et al. (2003). Oxidative stress mediated down regulation of bcl 2 promoter in hippocampal neurons, Wiley Online Library. 84: 982-996.

Saravanan, G. and Prakash, J. (2004). Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. J.Ethinopharmacol., 94: 155-158.

Saunders, J. A. and Conn E. E. (1978). "Presence of the cyanogenic glucoside dhurrin in isolated vacuoles from Sorghum." Plant physiology 61(2): 154.

Shati, A., Elsaid, F. G. and Hafez , E. E. (2011). Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup. Neuroscience.175, 66-74.

Schlorff, E. C., Husain, K. and Somani, S. M. (1999): Dose- and time-dependent effects of ethanol on plasma antioxidant system in rat. Alcohol, 17: 2, 97–105.

Shou, Y., N. Li, et al. (2002). NF B mediated up regulation of Bcl XS and Bax contributes to cytochrome c release in cyanide induced apoptosis, Wiley Online Library. 81: 842-852.

Slater,A.F., Stefan, C., Nobel, I., van den Dobblesteen, D.J. and Orrenius, S. (1995). Signalling mechanisms and oxidative stress in apoptois. Toxicol. Lett. 82–83, 149–153.

Sousa, A. B., Soto-Blanco, B. et al. (2002). "Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? Toxicology 174 (2): 87-95.

Sousa, A.B., Soto-Blanco, B. Guerra, J.L. Kimura E.T. and G´orniak, S.L. (2002). Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? Toxicology, 174: 87-95.

Sun, Y. and Oberley, L. W. The inhibition of catalase by glutathione.*Free Radical Biol. Med.* 7:595-602; 1989.

Towill, L.E., Drury, J.S., Whitfield, B.L., Lewis, E.B., Galyan E.L. and Hammons, A.S. (1978). Reviews of the Environmental Effects of Pollutants. V. Cyanide. U.S. Environ. Protection Agen., Rep. 600/1-78-027. 191.

Towill, L.E., Dury, J.S.,Whitfield, B.L., Lewis, E.B., Galyan, E.L., and Hammons, A.S. (1978). Reviews of the Environmental Effect of Pollutant Cyanide. Environmental Protection Agency Report No. 600/1-78-027, Ohio.

Tsujimoto, Y., Shimizu, S., Eguchi, Y., Kamiike, W. and Matsuda, H. (1997). Bcl-2 and Bcl-xL block apoptosis as well as necrosis: possible involvement of common mediators in apoptotic and necrotic signal transduction pathways. Leukemia 11 Suppl 3, 380–382.

Tulswani, P.K., Debnath, M., Pant, S.C., Kumar, O., Prakash, A.O., Vijayaraghavan R. and Bhattacharya, R. (2005). Effectsof sub-acute oral cyanide administration in rats. Protective efficacy of alphaketoglutarate and sodium thiosulphate. Chemico-Biological Interactions 156: 1-12.

Tylleskar, T., Banea, M. Bikangi, N., Fresco, L., Persson, L.A. and Rosling, H. (1991). Epidemiologicalevidence from Zaire for a dietary etiology of konzo, an upper motor neuron disease. Bull. WHO, 69, 581-589.

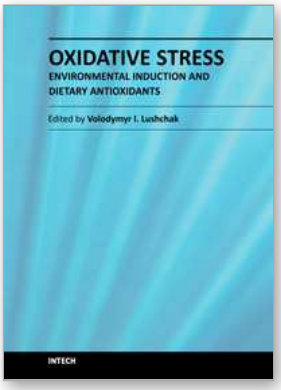
Watts, J. (1998). Japanese fear over festival cyanide poisoning. Lancet, 352-379.

Yen, D., J. Tsai, W. Kao, S. Hu, C. Lee and Deng, J. (1995). The clinical experience of acute CN- poisoning. Amer. J. Emergency Med., 13, 524-528.

Zhang, L., Li, L., Liu, H., Prabhakaran, K., Zhang , X., Borowitz J.L. and Isom G.E. (2007). HIF-1alpha activation by a redox-sensitive pathway mediates cyanide-induced BNIP3 upregulation and mitochondrial-dependent cell death. *Free Radic Biol Med* 43,117–127.

Zhang, X., L. Li, et al. (2009). Cyanide-induced death of dopaminergic cells is mediated by uncoupling protein-2 up-regulation and reduced Bcl-2 expression, Elsevier. 238: 11-19.

Zollner, N. and K. Kirsch (1962). "Microdetermination of lipids by the sulphophospho vanillin reaction." Z. Ges. Exp. Med 135, 545-561.



Oxidative Stress - Environmental Induction and Dietary Antioxidants

Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0553-4
Hard cover, 388 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Fahmy G. Elsaid (2012). Modification by Aqueous Extracts of Allium kurrat L. and Ricinus communis L. of Cyanide Nephrotoxicity on Balb/C Mice, Oxidative Stress - Environmental Induction and Dietary Antioxidants, Dr. Volodymyr Lushchak (Ed.), ISBN: 978-953-51-0553-4, InTech, Available from: http://www.intechopen.com/books/oxidative-stress-environmental-induction-and-dietary-antioxidants/modification-by-aqueous-extracts-of-allium-kurrat-l-and-ricinus-communis-l-on-cyanide-nephrotoxici

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.