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Protective Effects of *Allium kurrat* and *Ricinus communis* against Cyanide-Induced Hepatotoxicity in Balb/C Mice

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Abstract: This study addresses biochemical and molecular changes associated with cytotoxicity induced by cyanide in the liver of Balb/C mice. In addition, the protective effect of aqueous extracts of *Allium kurrat* and *Ricinus communis* against Cyanide induce hepatotoxicity will be investigated. About 40 male Balb/C mice (35-40 g) were used in this study and divided into 4 groups (10 mice each); control group, received no treatment; cyanide group, administered with potassium cyanide (KCN) in drinking water at 4.5 mg kg b.w. daily for 30 days; cyanide and *A. kurrat* group, co-administered with 4.5 mg/kg b.w./day of KCN and 200 mg/kg b.w./day of *A. kurrat* aqueous extract for 30 days. Cyanide and *Ricinus communis* group, co-administered with 4.5 mg/kg b.w./day of KCN and 200 mg/kg b.w./day of *R. communis* aqueous extract for 30 days. Oxidative stress, antioxidant status and liver function markers were estimated in the liver. The expression levels of *P53*, *Bcl-2*, Interleukin 4 (*IL-4*) and Interleukin 12 (*IL-12*) genes were examined using quantitative real time PCR technique. When compared with the control group, livers of the cyanide group showed a significant decrease in enzymatic antioxidant activities such as Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GSH-Red), Glutathione Peroxidase (GSH-Px) and in the non-enzymatic antioxidant such as Glutathione (GSH) content. The level of liver Thiobarbituric Acid Reactive Substances (TBARS) showed a signifcant increase of Lipid Peroxidation (LPO) in the cyanide group compared with controls. The liver function markers such as Aspartic Transaminase (AST) and Alanine Transferase (ALT) and total bilirubin increased in the cyanide group compared to control group. A significant increase in serum total cholesterol, total lipids and total protein was observed in cyanide group in coparasion with controls. However, treating those animals exposed to cyanide by *A. kurrat* and *R. communis* extracts alleviated the changes in all measured parameters. *P 53*, *Bcl-2*, *IL-4* and *IL-12* genes showed over expression in response to cyanide toxicity showed over-expression in the liver of the cyanide group compared to control. However, the *A. kurrat* and *R. communis* extracts were able to manage the molecular changes induced by cyanide. It can be concluded that extracts of *A. Kurrat* and *R. communis* have promising role of for the treatment of cyanide induced hepatotoxicity.

Key words: Hepatotoxicity, cyanide, antioxidant enzymes, liver functions, real-time PCR, Balb/C mice, *Allium kurrat*, *Ricinus communis* L.

INTRODUCTION

Cyanide is an environmental toxic substance which has been associated with many intoxication cases in humans and animals resulting from the ingestion of foods and exposure to environmental pollution, chemical war, fertilizers, pesticides, insecticides and use in some drugs (Sousa *et al*., 2002). In addition, cyanide can be found in many plant species such as *Manihot* sp. (cassava), *Linum* sp., *Lotus* sp., *Phaseolus lunatus*, *Sorghum* sp., as cyanogenic glycosides to defend the plant against herbivores (Nhassico *et al*., 2008). Exposure to cyanide increases its concentration in erythrocytes through binding to methemoglobin and causes hypothyroidism that leads to goiter (Elsaid and Elkomy, 2006). Sub-acute oral administration of cyanide in rats produced changes in several biochemical indices and pathology in various organs (Tulsawani *et al*., 2005).

Cyanide-induced cellular oxidative stress aears 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). Aerobic organisms have antioxidant defense systems that deal with Reactive Oxygen Species (ROS) (Fukai *et al*., 2002). Superoxide Dismutase (SOD) catalyzes the conversion of the highly reactive superoxide anion to O_2 and to H_2O_2 (Fukai *et al*., 2002). This less damaging molecule can be converted spontaneously to highly reactive hydroxyl radicals. However, Catalase

(CAT) and Glutathione Peroxidase (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 (Tulsawani *et al*., 2005). Levels of Malondialdehyde (MDA), GSH and GSSH are also correlated with lipid peroxidation (Tulsawani *et al*., 2005). The decrease of cellular GSH following exposure to cyanide has been attributed, in part to reduced cellular ATP resulting from inhibition of cytochrome c oxidase (Prabhakaran *et al*., 2006). Antioxidants can block the enhanced apoptosis produced by cyanide and this could be linked to generation of ROS (Jones *et al*., 2003).

It has been reported that mild damage to mitochondrial and/or cellular membranes of the liver indicated with high AST activity after intake of cassava (Nhassico *et al*., 2008). Changes in AST activities of rats that received KCN (9 mg kg^{-1} b.w.) which could be related to hepatic lesion was verified (Sousa *et al*., 2002). The degenerative changes manifested in the liver of the cyanide-fed rabbits are consistent with the observed pattern of activities of ALT and Alkaline Phosphatase (ALP) in the serum and tissues of these animals (Elsaid and Elkomy, 2006; Sousa *et al*., 2002).

Gene expression was deflected by cyanide intoxication. 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 (Han *et al*., 2008). The loss of p53 function activates oncogenes and inactivates cancer suressor genes, playing an essential role in multistage carcinogenesis (Vogelstein *et al*., 2000). Bcl-2 is constitutively expressed and localized to the outer mitochondrial membrane where it attenuates cell death signals to promote cell survival (Reed, 2000). Bcl-2 exerts an anti-apoptotic effect by inhibiting mitochondrial outer membrane permeabilization to suress release of cytochrome c into the cytosol (Cory and Adams, 2002). 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 (Denecker *et al*., 2001). Forced over-expression of Bcl-2 can block cell death produced by a variety of stimuli, including cyanide (Zhang *et al*., 2007). Over-expression 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 injury models, IL-4 has been shown to be both protective and deleterious. IL-4 accelerates severe hepatitis in mice deficient in suressor 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 (Dong *et al*., 2007).

Hepatic system has been reported to be resistant to cyanide due to the presence of high levels of rhodanese enzyme that carries out the biotransformation of cyanide (Mathangi *et al*., 2011). 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 alications where a longer duration of action is required (Rockwood *et al*., 2003). Cyanide detoxification occurs *in vivo* mainly by conversion to thiocyanate. This reaction is catalyzed by the enzyme rhodanese. In the presence of excess cyanide, the rate-limiting step is an adequate suly of sulphane sulphur (a divalent sulphur atom bonded to another sulphur atom) which can be sulied by thiosulphate (Vale, 2007). However, the principal detoxification pathway of cyanide to thiocyanate in the presence of sulfur donor like sodium thiosulphate is mainly catalyzed by a liver mitochondrial enzyme, rhodanese (Cyanide:thiosulphate sulphur transferase) (Hildebrandt and Grieshaber, 2008). As the Egyptian leek, *A. kurrat*, contains gallic acid as a phenolic component and vitamin C, it has antioxidant properties (Souzan and El-Aal, 2007). In addition, the leaves of the castor oil plant *R. communis* has been found to contain flavanoids like kaempferol-3-0-β-D-rutinoside and kaempferol-3-0-β-D-xylopyranoid and tannins (Lin *et al*., 2000; Atta and Mouneir, 2005). Therefore, the present study aims to investigate the antidotal effect of the aqueous extracts of *A. kurrat* and *R. communis* against cyanide hepatotoxicity. In addition, this study aimed to investigate the genotoxic effect of cyanide on the liver tissues and the ameliorative effect of the aqueous extracts of *A. kurrat* and *R. communis* against this toxicity.

MATERIALS AND METHODS

Natural products extracts: *A. kurrat* (Egyptian leek), family Alliaceae was purchased from the local supermarket in Abha, Saudi Arabia. *R. communis* (Castor), family Euphorbiaceae was collected from Asir region, Saudi Arabia. The leaves of *A. kurrat* and *R. communis* were separately immersed in distilled water for 24 h, filtered, stored at -20°C and freshly prepared every 3 days.

Animal grouping: A total of 20 mice weighed (45 g) were used in and obtained from the animal house at College of

Science, King Khalid University, Saudi Arabia. The local committee aroved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH). The mice were provided with *ad libitum* access to water and a standard chow diet and divided into 4 groups, 5 each: control group, mice received no treatment; cyanide group, mice were administered with 4.5 mg/kg b.w./day of KCN (0.5 of LD_{50}) in drinking water (Sousa *et al*., 2002) for 30 days, freshly prepared every 4 days; cyanide and *A. kurrat* group, mice were co-administered with 4.5 mg/kg b.w./day KCN in drinking water for 30 days and intraperitoneally administered *A. kurrat* extract at 200 mg/kg b.w./day; cyanide and *R. communis* group, mice co-administered with 9 mg/kg b.w./day cyanide and intraperitoneally administered with *R.* extract at 200 mg/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 liver tissue were collected from each group. The liver tissues were homogenized in a phosphate buffer solution pH 7.4, centrifuged at 4°C and the supernatant was stored at -80°C.

Enzymatic assays: Superoxide Dismutase (SOD) activity was determined by the method of Nishikimi *et al*. (1972) in which the inhibition of formation of NADPH-phenazine metho-sulphate nitroblue tetrazolium formazon was measured spectrophotometrically at 560 nm. Catalase (CAT) activity was assayed colorimetrically as described by Aebi (1984). Glutathione Peroxidase activity (GSH-Px) was assayed by the method based on the reaction between glutathione remaining after the action of GSH-Px and 5, 50-dithiobis-2-nitrobenzoic acid to form a complex that absorbs maximally at 412 nm (Paglia and Valentine, 1967). Glutathione Reductase (GSH-Red) that utilizes NADPH to convert metabolized Glutathione (GSSG) to the reduced form was assayed by the method of Mize and Langdon (1962). Glutathione (GSH) serves 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. GSH content was assayed by 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 405 nm (Ellman, 1959). Determination of 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 will lack a certain amount of the provided hydrogen peroxide. The residual H_2O_2 is 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 (LPO): The LPO in the liver was measured by the quantification of TBARS and determined by the method of Buege and Aust (1978).

Liver function markers: Activities of Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST) in sera were assayed according to Reitman and Frankel (1957).

Total bilirubin, total lipid, serum cholesterol and total protein: Bilirubin was measured by the method of Walter and Grade (1970). Total lipid according to the method of Zollner and Kirsch (1962). Liver total protein was determined by the methods of Gornall *et al*. (1949) and serum cholesterol was measured using the method of Richmond (1973).

Extraction of total RNA from liver tissue homogenates: Total RNA was isolated from liver 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).

Real time PCR and gene expression: For *P53* and *Bcl-2* genes; the extracted RNA from liver tissues of different groups was subjected to examine the expression level of 2 genes using specific primers in the presence of housekeeping gene primers. The real time reaction consists of 12.5 μL of 2X Quantitech SYBR® Green RT Mix (Fermentaz, USA), 2 μL of the extracted RNA (50 ng^{-1} μL), 1 μL of 25 pM μL^{-1} Forward (F) primer, 1 μL of 25 pM μL^{-1} 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). 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 liver 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.

Molecular data analysis: Comparative quantitation analysis was performed using Rotor-Gene-6000 series software based on the following equation:

$$\text{Ratio target gene expression} = \frac{\text{Fold change in target gene expression}\left(\frac{\text{Sample}}{\text{Control}}\right)}{\text{Fold change in reference gene expression}\left(\frac{\text{House keeping gene}}{\text{Control}}\right)}$$

Real-time PCR data of all samples were analyzed with aropriate 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.

Statistical analysis of biochemical data: The biochemical data recorded were expressed as mean±SD and statistical and correlation analyses were performed using the one-way ANOVA followed by the post-hoc LSD (Least Significant Difference) test. $p \leq 0.05$ were considered as statistically significant. Statistical analyses were performed with the Statistical Package for the Social Sciences for Windows (SPSS, Version 10.0, Chicago, IL, USA).

Table 1: Oligonucleotide primer sequences used in this study

Primer names	Primer sequence from 5`-3`	Annealing temperature
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	60-65°C

GPDH: Glucose 6-Phosphate Dehydrogenase

RESULTS AND DISCUSSION

There was a significant decrease in the activity of the total antioxidant capacity, CAT, SOD, GSH-PX, GSH-Red and GSH content in the liver homogenates of the cyanide groups when compared with their matched control ones (Table 2). Moreover, there was a significant increase in lipid peroxidation as shown by the increase in the TBARS of the cyanide group livers (Table 2). All changes in biochemical parameters in liver tissues were alleviated in Cyanide and *A. kurrat* and cyanide and *R. communis* groups (Table 2).

There was a significant increase in serum total billirubin, total cholesterol, total lipids, total protein, AST and ALT levels in cyanide group in comparison with controls (Table 3). However, the aqueous extracts of *A. kurrat* and *R. communis* could alleviate the disruption in these parameters (Table 3).

Molecular data: Expression of P53 was significantly increased in the cyanide group when compared with control group (Fig. 1).

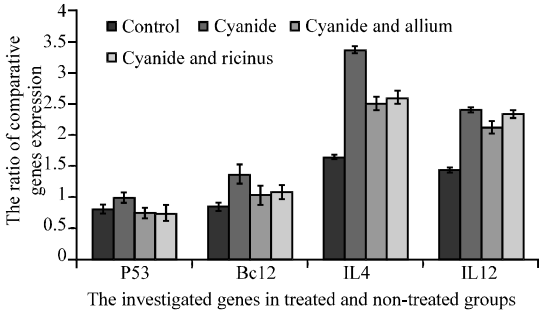

Fig. 1: The expression of different genes in the liver of treated and non-treated groups

Table 2: Liver antioxidant activity in treated and non-treated Balb/C groups

	Groups			
Parameters	Control	Cyanide	Cyanide and *A. kurrat*	Cyanide and *R. communis*
T. anti-oxidant capacity (U g^{-1} tissue)	0.56±0.05	0.31±0.06***	0.44±0.03***†	0.49±0.03*†
CAT (U g^{-1} tissue)	6.80±0.63	4.50±0.77***	5.50±0.65**†	6.10±0.34†††
SOD (U g^{-1} tissue)	58.60±4.80	44.20±3.90***	44.30±2.90***	56.50±4.50†††
GSH-PX (U g^{-1} tissue)	5.80±0.64	3.50±0.63***	4.30±0.43***†	5.30±0.51†††
GSH-Red (U g^{-1} tissue)	73.10±3.10	48.50±4.60***	52.70±4.20***	61.20±3.42***†††
GSH (U g^{-1} tissue)	6.60±0.48	4.50±0.82***	4.80±0.77***	5.80±0.87††
TBARS (nmole g^{-1} tissue)	100.10±6.90	230.40±24.6***	149.90±19.0***†††	139.60±13.2**†††

All groups were compared with the control group; *p<0.05, **p<0.01, ***p<0.001; Cyanide and *A. kurrat* and Cyanide and *R. communis* groups were compared with cyanide group; †p<0.05, ††p<0.01, †††p<0.001

Table 3: Liver function parameters in treated and non-treated Balb/C groups

	Groups			
Parameters	Control	Cyanide	Cyanide and *A. kurrat*	Cyanide and *R. communis*
Serum bilirubin	0.95±0.21	2.95±0.85***	2.58±0.5***	1.89±0.29**††
Serum AST	208.8±20.9	275.4±17***	266.7±19.5***	242.2±14.1**††
Serum ALT	70.1±4.9	99±14.4***	91.5±7.4**	77.6±8.6††
Serum T. Cholesterol	100.9±15.7	73.8±5.7***	75.5±6.9***	92.9±9.4††
Liver T. Lipids	32.6±4.6	21.6±3.1***	27.6±3.5*†	32.4±5.1†††
Liver T. Protein	10.1±0.98	5.3±0.81***	6.4±1***	6.9±1.5***†

All groups were compared with the control group; *p<0.05, **p<0.01, ***p<0.001; Cyanide and *A. kurrat* and Cyanide and *R. communis* groups were compared with cyanide group; †p<0.05, ††p<0.01, †††p<0.001

However, expression of *P53* gene was decreased in *A. kurrat* and *R. communis* groups when compared with cyanide group (Fig. 1). *Bcl-2* gene was highly expressed in cyanide intoxicated mice in comparison with controls. However, expression of *Bcl-2* gene was decreased in *A. kurrat* and *R. communis* groups when compared with cyanide group (Fig. 1).

The expression of *IL-4* and *IL-12* genes was higher in cyanide group when compared with controls (Fig. 1). However, expression of these genes was decreased in *A. kurrat* and *R. communis* groups when compared with cyanide group (Fig. 1).

Acute cyanide intoxication mainly occurs due to cytotoxic anoxia following inhibition of cytochrome oxidase, a respiratory chain enzyme (Sousa *et al*., 2002). Although, cyanide is mainly a neurotoxin, involvement of liver and kidney in cyanide toxicity has also been reported (Sousa *et al*., 2002). It is well characterized that cyanide inhibits cytochrome oxidase which in turn stimulates ROS formation at complexes 1 and 3. 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 (Ott *et al*., 2007).

Lipids, proteins and DNA are all susceptible to attack by free radicals. The oxidative stress induced by cyanide in this study, as evidenced by elevation of liver lipid peroxidation associated with inhibition of antioxidants enzymes, suggests the participation of free radical-induced oxidative cell injury in mediating the toxicity of cyanide as proposed by other studies (Daya *et al*., 2000; Hariharakrishnan *et al*., 2009). In addition, the cyanide-induced oxidative stress leads to a decrease in GSH content, GSH-PX, GSH-Red activities. It exerts its effect by enhancing the non-enzymatic antioxidant as GSH and the detoxifying enzyme such as glutathione S-transferase (Saravanan and Prakash, 2004). Therefore, it restores glutathione level and increases the activities of glutathione reductase and Glutathione-S-Transferase (GST) (Saravanan and Prakash, 2004). GST is a detoxification enzyme which catalyzes the conjugation of many electrophilic agents with GSH (Hayes *et al*., 2005) and hence, it may be bind to cyanide which may explain their decrease in liver tissues of cyanide intoxicated mice. In agreement with previous studies (Tulsawani *et al*., 2005), GSH, an intracellular antioxidant was depleted after exposure to cyanide making the cell environment vulnerable to oxidative stress. In the present study, changes in liver function parameters such as AST and ALT activities and total billirubin levels of rats in the cyanide group could be related to hepatic lesion as proposed by other studies (Elsaid and Elkomy, 2006; Sousa *et al*., 2002). The observed increase in the levels of cholesterol or oxidatively modified lipoproteins in blood circulation could be associated with mitochondrial dysfunction as proposed by other studies (Puddu *et al*., 2005).

Organosulphurs enhance the synthesis of the cellular GSH content (Wu *et al*., 2001) which is catalyzed by antioxidant enzymes such as gamma-glutamyl transpeptidase (Lu, 2008) and it may also alleviate the disrupted liver function enzymes. The protective efficacy of *A. kurrat* (Souzan and El-Aal, 2007) and *R. communis* (Ilavarasan *et al*., 2006) against cyanide-induced cytotoxicity can be attributed to their antioxidant properties of both. Also, it can be interpreted by the sulphur donor properities of *A. kurrat* (Souzan and El-Aal, 2007).

Physiological effects of cyanide may be mediated through changes in the expression of gene products, either at the mRNA or protein levels. The increased expression of p53, tumor suressor gene and bcl-2, apoptosis regulating gene indicates that liver cells apoptosis and programmed cell death were triggered by cyanide intoxication either due to oxidative stress or DNA damage (Han *et al*., 2008). However, extracts of *A. kurrat* and *R. communis* could lower the enhanced expressions of *p53* and *bcl-2* genes. This indicates that they were able to scavenge cells from oxidative stress, as evidenced by alleviating increased LPO in this study and hence, cells may not directed to apoptosis. Currently, it is generally believed that the role of IL-4 in Con A-mediated hepatitis

is mediated via enhancing the cytotoxicity of natural killer T-cells against hepatocytes (Dong *et al*., 2007). Interleukin-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 (Rentzos *et al*., 2009). Cyanide enhanced the expression of *IL-4* and *IL-12* encoding genes indicating that the immune system of mice intoxicated with cyanide was affected. However, treating cyanide intoxicated mice with extracts of *A. kurrat* and *R. communis* could lower the expression of these genes. This indicates that both *A. kurrat* and *R. communis* extracts could help in increasing the immune system of intoxicated mice through modulating the interleukins expression as anti-inflammatory.

CONCLUSION

The toxicity of cyanide associated with decreased in the antioxidant enzymes with increased in LPO the liver of Balb/C mice. *P53*, *Bcl-2*, *IL-4*, *IL-12* genes showed over-expression in the liver of cyanide intoxicated mice. However, extracts of *A. kurrat* and *R. communis* could alleviate all biochemical and molecular changes associated with cyanide hepatotoxicity.

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