

Antioxidant Effects of Methanol Extract of *Allium cepa linn* on Cyanide-induced Renal Toxicity in Male Wistar Rats

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Summary: The protective effects of onion was assessed in the Kidney of rats following sub-acute exposure to cyanide. These effects were compared to those of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), a classical antidote of cyanide toxicity. The rats were divided into 6 groups of 6 animals each. Group 1 was administered distilled water, Group 2,3,4, 5, and 6 were administered 600 mg onion/kg bwt/day, 7 mg KCN/kg bwt/day, 300 mg onion/kg bwt/day+ 7 mg KCN/kg bwt/day, 600 mg onion/kg bwt/day+ 7mgKCN/kg bwt/day, 600mg $\text{Na}_2\text{S}_2\text{O}_3$ /kg bwt/day+ 7mgKCN/kg bwt/day respectively for 2 weeks. Group 3, 4 and 5 were pre-administered with 300mg onion/kg bwt/day, 600mg onion/kg bwt/day and 600mg $\text{Na}_2\text{S}_2\text{O}_3$ /kg bwt/day respectively for 2 weeks. Serum and urine creatinine and urea level were assessed as a measure of kidney function. Oxidative stress and antioxidant parameters were estimated in Kidney. Serum creatinine and urea levels were significantly higher in the cyanide treated rats compared with control. This was accompanied by significant reduction in the urine level of creatinine and urea. Co-administration with onion extract and $\text{Na}_2\text{S}_2\text{O}_3$ reverse the situation in both the serum and urine. The level of malondialdehyde (MDA) in rats treated with cyanide ($3.846 \pm 0.20 \mu\text{g/g}$) was significantly increased in the kidney relative to control ($0.691 \pm 0.15 \mu\text{g/g}$). This was accompanied with a decreased in antioxidant enzymes Superoxide Dismutase (SOD) ($2.0 \pm 0.09 \text{U/mg}$), Catalase (CAT) ($0.014 \pm 0.001 \text{katf}$), Glutathione-S-Transferase (GST) ($0.015 \pm 0.009 \text{nMol/mg}$) and non-enzymatic antioxidant Reduced Glutathione (GSH) ($4.006 \pm 0.09 \mu\text{g/ml}$) compared with control ($4.8 \pm 0.13 \text{U/mg}$, $0.047 \pm 0.001 \text{katf}$, $0.022 \pm 0.0013 \text{nMol/mg}$, $6.802 \pm 0.2 \mu\text{g/ml}$ respectively). Co-administration with onion extract and $\text{Na}_2\text{S}_2\text{O}_3$ significantly increased these antioxidant enzymes and significantly decreased the concentration of malondialdehyde in the kidney. The results indicate that onion extract reduced lipid peroxidation in the kidney and increased antioxidant status of animals exposed to cyanide in a dose dependent manner

Keywords: Onion, Sodium thiosulphate, Lipid peroxidation, Antioxidant, Cyanide.

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Manuscript Accepted: December 2014

INTRODUCTION

Cyanide is a serious environmental pollutant that is extremely toxic to several forms of life because of its inhibitory activity on a variety of key enzymes (Cipollone et al., 2007). One major enzyme inhibited by cyanide is the cytochrome c oxidase enzyme, in which it prevents cellular oxygen utilization and ATP production (Way, 1984).

Cyanide poisoning may result from exposure to hydrocyanic acid and cyanide salts (Benaissa et al, 1995). Cyanide is found in a number of plants such as cassava roots, in the form of cyanogenic glycosides (Vetter, 2000). In cases of fatal oral poisoning cyanide has been detected in the brain, blood, liver and also kidney (Ansell and Lewis, 1970).

The mechanisms by which cyanide exert its effects have not all been fully elucidated. Although in the inhibition of the mitochondria respiratory chain, cyanide produces the superoxide anion. Cyanide has been shown to induce oxidative stress (lipid peroxidation) in the brain of acutely treated mice (Mills et al., 1996). Some of its biochemical effects could therefore be mediated through the generation of

reactive oxygen species on some target organs such as the kidney (Okafor et. al., 2006). Exposure to high levels of ROS leads to depletion in antioxidant levels in animals. One of these antioxidants in the body is reduced glutathione, a reducing agent in biological cells that provides primary antioxidant defence against reactive intermediates of metabolism, drugs or carcinogens (Meister and Anderson, 1983). Cyanide is also a potent inhibitor of the enzymatic antioxidants catalase, superoxide dismutase, and glutathione peroxidase (Kanthasamy et al, 1997). These mechanisms may function in concert to produce the oxidative stress and damage seen after cyanide exposure.

Allium cepa linn (onion), is used as foodstuff, condiments, flavouring, and in folk medicine. Onions have been extensively studied for their therapeutic uses as antibiotic, antidiabetic, antiatherogenic, anticancer etc (Augusti, 1996). Onion oil has been reported to effectively decrease the lipid levels in experimental animals (Bobbi et al., 1984). It has been found that administration of onion products to diabetic rats significantly reduced hyperglycaemia (Kumud et al., 1990). Biological action of *Allium*

products is ascribed to organosulfur compounds, which have also been shown to possess antioxidant and free radical scavenging activities. Onions have previously been shown to protect testis against cadmium induced oxidative stress in rats (Ola-Mudathir et al., 2008). It is therefore hypothesized that onion extract could exert a protective effect against cyanide induced oxidative stress in the kidney.

Thus, this study investigates the protective effects of onion against cyanide induced oxidative stress in the kidney of wistar rats.

MATERIALS AND METHODS

Plants collection and preparation of extracts

Fresh bulbs of red onions were collected from Bodija market, Ibadan Nigeria and authenticated in the Department of Botany, University of Ibadan.

Extraction of plant material

The onions were washed with distilled water and allowed to air dry for one hour. The outer covering of the onion were manually peeled off. The onion bulbs being separated were washed and extracted in the following ways: Exactly 786g of fresh onion bulbs were blended on ice and soaked in 1000 ml of absolute methanol for 72hr in a clean, glass container and was filtered using a sterile muslin cloth after which the extract obtained was dried using the rotatory evaporator and stored in a refrigerator until required.

Experimental procedure

Thirty six male albino rats obtained from the animal colony of the Central Animal House of University of Ibadan were used. All animals were kept at room temperatures and had free access to drinking water and their pellets. The animals were acclimatized for two weeks to their environment before experimentation. All animal experiments were conducted in accordance with the International Ethical Norms on Animal Care and Use as contained in NIH publication/85-23, revised in 1985.

The rats were grouped into six groups (1, 2, 3, 4, 5 and 6) of six rats each. Group 1 served as control and received 0.2ml /day distilled water. Group 2 animals were treated orally with methanolic extract of *Allium cepa* (600mg/kg bwt/day). Group 3 was treated orally with potassium cyanide (7mg/kg bwt). Group 4 animals were treated orally with methanolic extract of *Allium cepa* (300 mg/kg bwt/day) and potassium cyanide (KCN) (7mg/kg bwt/day). Group 5 was treated orally with aqueous extract of *Allium cepa* 600 mg/kg bwt/day + 7mg/kgbw/day KCN. Group 6 was treated orally with thiosulphate 600mg/kg bwt/day + 7mg/kg bw/day KCN. Group 4, 5 and 6 were pre-administered with 300mg onion/kg bwt/day,

600mg onion/kg bwt/day and 600mg Na₂S₂O₃ /kg bwt/day respectively for 2 weeks before co-administration of onion and potassium cyanide. The animals were observed daily and weighed at intervals of 2 days for two weeks. At the end of the experiments, the animals were fasted overnight and sacrificed by cervical dislocation. Blood samples were collected into plain tubes and centrifuged at 1500g for 10minutes to obtain serum for biochemical analysis. The kidneys were removed into ice-cold container for evaluation of oxidative stress and antioxidant enzymes. Tissue sample for histology was fixed in 10% saline formalin.

Biochemical analysis

Level of Lipid peroxidation was evaluated by method of Buege and Aust (1978). Catalase activity was evaluated by method of Ashru and Sinha (1971). SOD activity was evaluated by method of Misra and Fridovich (1972). GST activity was evaluated by the method of Habig et al (1974). Protein estimation was done by method of Lowrey (1951). GSH content was evaluated by the method of Beutler et al (1963). Serum and urine creatinine and urea were evaluated using Randox® creatine and urea kits, respectively.

Histology

Histology of Kidney was done using H and E staining techniques.

Statistical Analysis

Data were presented as Mean ± SEM Student t test and ANOVA were used for comparison of results. P< 0.05 was considered significant.

RESULTS

Relative tissue weight

Relative kidney weight was significantly higher in the cyanide treated group (P<0.05) when compared to the control and co-administration of onion and Sodium thiosulphate did not produce any significant change (Table1).

Serum and urine analyte

Serum urea and creatinine level was significantly increased in the cyanide treated group (p<0.001) compared to control and this was reduced significantly by Co-administration with onion and sodium thiosulphate (p<0.001), while Urine creatinine level was significantly lowered in the cyanide treated group (p<0.001) compared to control. Co-administration with onion and sodium thiosulphate, however significantly increase it (p<0.001) (Table 3). Urine urea level was significantly lower in the cyanide treated group (p<0.001) compared to control and it was raised significantly by Co-administration of onion extract and sodium thiosulphate (p<0.001) (Table 1).

Oxidative stress Marker in the Kidney

Kidney malondialdehyde were significantly higher in the cyanide treated group (P<0.001) compared to the control and were reduced significantly by co-administration with onion extracts and sodium thiosulphate (p<0.001). Kidney SOD activities was significantly lowered in the cyanide only group compared to control (p<0.001) and was significantly increased by co-administration with the onion extracts (p<0.001). Co-administration of sodium thiosulphate however does not have any significant affect (Table 2).

Catalase activities in the kidney was significantly lowered in the cyanide only group compared to control (p<0.001) and was significantly increased by co-administration with the onion extracts and Sodium thiosulphate (p<0.001) (Table 2). Kidney GST activities was significantly lower in the cyanide only group compared to control (p<0.01) and co-administration with 600mg/kg bwt onion extract and sodium thiosulphate significantly increase it (p<0.01) (Table 2). Kidney GSH activities was significantly reduced in the cyanide only group with respect to the control (p<0.01) and was increased significantly by co-administration with 600mg/kg bwt onion extract and sodium thiosulphate (P<0.01), co-administration with 300mg/kg bwt produced no significantly affect (Table 2).

Histopathology of the Kidney

As shown in Figure 1A, B and E, kidneys of the control, onion extract only and 600mg/kg bwt onion co-administered with cyanide treated animals had intact cyto-architecture, glomeruli and collecting ducts. Fig 1C shows acute tubular necrosis for cyanide only group. Fig 1D and F also shows normal glomeruli with very mild focal tubular necrosis for 300mg/kg bwt onion extract and sodium thiosulphate co-administered with cyanide group respectively.

DISCUSSION

The present study shows that sub-acute cyanide toxicity increased the serum urea and serum creatinine level. One major function of the kidney is clearance of metabolites from the blood (Guyton and Hall, 2006). Agents with nephro-toxicity effects may raise serum creatinine and urea level. In addition

cyanide decreased the urine creatinine and Urea level, suggesting that glomerular filtration rate might have been impaired. However, these effects were ameliorated by co-administration of onion extract or Na₂S₂O₃.

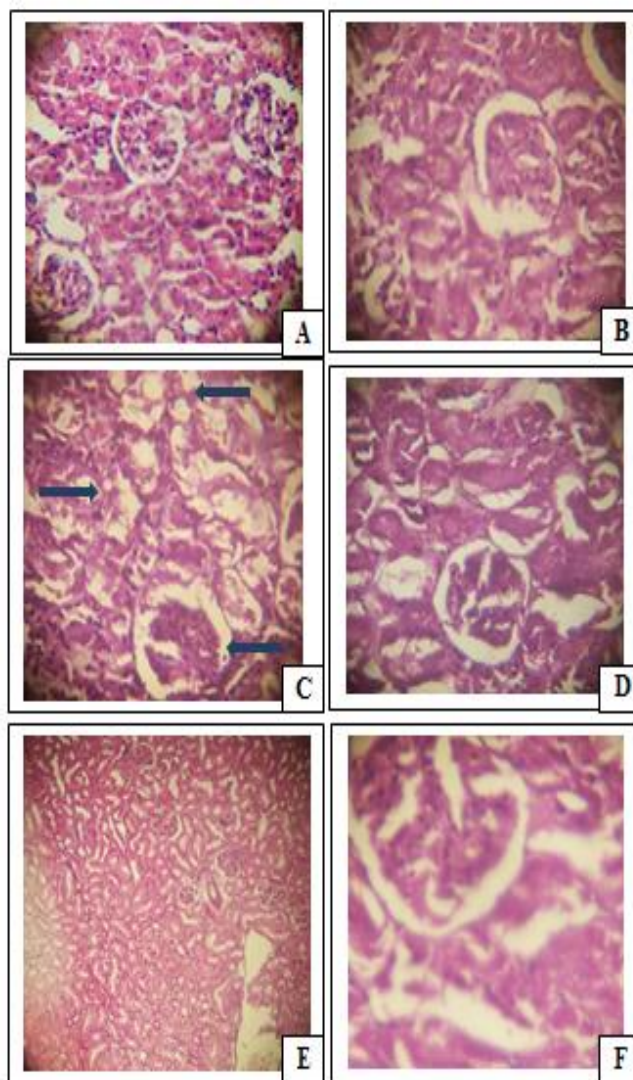


Fig 1. Photomicrograph showing transverse section of the kidney in (A) control animals (B) Allium cepa only (C) CN only treated with acute tubular necrosis (D) 300mg Allium cepa + KCN with mild focal tubular necrosis (E) 600mg Allium cepa + KCN (F) Sodium Thiosulphate + KCN with mild focal tubular necrosis.

Table 1: Effects of methanol extract of *A. Cepa* on relative organ weight and serum and urine creatinine and urea.

Groups	Relative Organ wt	S _{creatinine} (μ mol/l)	U _{creatinine} (mmol/l)	S _{urea} (mmol/l)	U _{urea} (mmol/l)
1	6.07±0.14	73.19±3.37	1.515±0.004	4.618±0.40	9.476±0.30
2	6.02±0.21	74.21±2.14	2.31±0.4	5.120±0.30	8.760±0.31
3	6.65±0.19 *	134.53±2.73 *#	0.529±0.004 **	7.650±0.35 **	3.204±0.37 **
4	6.14±0.31	92.63±2.07	3.256±0.675	7.268±0.27	5.84±0.24
5	6.22±0.31	96.32±2.04	2.93±0.41	7.290±0.23	4.970±0.31
6	6.19±0.24	81.32±0.12	4.21±0.432	7.213±0.32	5.453±0.341

1= Control, 2=Onion Only, 3=Cyanide Only, 4=Cyanide+300mg onion extract, 5=Cyanide+600mgonion extract, 6=Cyanide+Sodium thiosulphate. S= Serum, U= Urine, *p< 0.05 (compared to control), #p<0.05(compared to Cyanide+600mg onion extract).

Table 2: Effects of methanol extract of *A. Cepa* on Kidney MDA, GSH, antioxidant enzymes and protein.

Groups	Kidney MDA ($\mu\text{g/g}$ tissue)	Kidney SOD (U/mg protein)	Kidney catalase (Katf)	Kidney GST (nmol/mg protein)	Kidney GSH ($\mu\text{g/ml}$)	Kidney Protein (mg/ml)
1	0.69 \pm 0.15	4.80 \pm 0.13	0.047 \pm 0.001	0.022 \pm 0.0013	6.802 \pm 0.2	1.678 \pm 0.03
2	0.48 \pm 0.09	4.40 \pm 0.14	0.050 \pm 0.0012	0.023 \pm 0.00129	6.956 \pm 0.1	1.96 \pm 0.028*
3	3.85 \pm 0.20*#	2.00 \pm 0.09*#	0.014 \pm 0.001*#	0.015 \pm 0.009*#	4.006 \pm 0.09*#	1.872 \pm 0.02*#
4	1.79 \pm 0.21*#	3.20 \pm 0.12*	0.030 \pm 0.004*	0.014 \pm 0.0012*#	4.64 \pm 0.2*#	1.632 \pm 0.02
5	0.67 \pm 0.17	4.40 \pm 0.10	0.040 \pm 0.003	0.021 \pm 0.001	6.594 \pm 0.1	1.596 \pm 0.014
6	2.16 \pm 0.15*#	2.80 \pm 0.76*#	0.025 \pm 0.005*#	0.018 \pm 0.001	4.994 \pm 0.1*#	1.354 \pm 0.015*#

1= Control, 2=Onion Only, 3=Cyanide Only, 4=Cyanide+300mg onion extract, 5=Cyanide+600mgonion extract, 6=Cyanide+Sodium thiosulphate. S= Serum, U= Urine, *p< 0.05 (compared to control), #p<0.05(compared to Cyanide+600mg onion extract).

Cyanide is a rapid-acting mitochondrial toxicant that inhibits cytochrome oxidase, thereby blocking the flow of electrons through complex IV to prevent oxidative metabolism (Jones et al., 2003) and enhance ROS generation at complex III (Chen et al., 2003). The damaging effects of cyanide on the tissue and organ could be mediated by cyanide ion (being a nucleophile) and its inhibition of mitochondria respiratory chain thus producing free radical such as superoxide anion (Mills *et al.*, 1996), resulting in lipid peroxidation. Thus some of the effects observed in cyanide treated rats could be mediated through attack of the generated ROS on some target organ and cells such as the, kidney. Also exposure to high levels of ROS leads to depletion in antioxidant levels in the animals resulting in oxidative stress. (Wu and Cederbaum, 2003). The results in this study also confirm that cyanide exposure increase malondialdehyde (a product of lipid peroxidation) concentration in the kidney treated rats.

The increased concentration of LPO products observed in the kidney of cyanide treated rats is also associated with decreased activity of scavenging enzymes such as catalase and superoxide dismutase in the kidney of these animals. A decrease in the activities of these enzymes can lead to the excessive availability of superoxides and peroxy radicals, which in turn generate hydroxyl radicals resulting in the initiation and propagation of LPO (Sacks et al., 1978). Earlier studies have shown that cyanide inhibit the antioxidant enzymes catalase, SOD, glutathione peroxidase (Kanthasamy et al., 1997), GST and reduces the level of GSH (Elsaid and Magedah, 2006).

Supplementation of onion extract and sodium thiosulphate to cyanide treated rats counteracts the increased levels of LPO products in the kidney. This decreased LPO is associated with increased activities of the antioxidant enzymes-catalase and SOD which was more pronounced in the 600mg/kg onion extract co-administered than in animals co-administered with 300mg/kg or 600mg/kg sodium thiosulphate. This indicates that treatment with onion extract is dose dependent and also more effective than treatment with sodium thiosulphate.

One of the antioxidants in the body is the reduced glutathione, a reducing agents in biological cells that

provide a primary antioxidant defence against reactive intermediates of metabolism, drugs or carcinogens (Meister and Anderson.1983). The present study also shows a decrease in the non-enzymatic antioxidant glutathione (GSH) content and the antioxidant glutathione-s-transferase (GST) activity in the kidney of cyanide treated rats, these may further contribute to the increase in LPO observed in these animals. This is in agreement with earlier studies in which ingestion of cassava cyanide depletes blood glutathione. This depletion in glutathione status could be one of the mechanisms by which cyanide exerts its numerous toxicities (Mcmillian and Geervarghese, 1979). Cysteine and glutathione derivatives are major constituents of onion (Lancaster and Kelly 1993), the presence of these compounds in onion could increase the non-enzymatic antioxidant (GSH) and the detoxifying enzyme (GST). These compounds have been shown to participate in s-thiolation process which affects the structure and functions of proteins. Thus onion could help reduce the damaging effect of cyanide by increasing the body's glutathione level and GST activity, as cyanide has been shown to deplete whole blood glutathione (Okafor et al 2008). Onions are also rich in flavonoid and phenolic which possess antioxidant properties (Lanzotti 2006).

Decreased LPO, high increased GST and GSH observed in sodium thiosulphate supplemented rats could also be attributed to the sulphur contained in it. While that of onion extract to its rich sulfur containing active compounds in the form of cysteine derivatives. It is known that onion oil contains dialkyl disulfides ($\text{CH}_3\text{-S-S-CH}_3$, $\text{CH}_3\text{-S-S-C}_3\text{H}_5$, $\text{CH}_3\text{-S-S-C}_3\text{H}_7$, etc.) and their oxides and thiols, which can trap electrons from other systems (Klanns-Dieter, 1983). Thus it prevents superoxide formation to a certain extent and scavenges many free radicals including hydroxyl radicals. Therefore the antidotal effect of onion on cyanide toxicity may be due to the fact that organosulphur contained in onion have antioxidant and detoxifying ability. These detoxifying effects are related to their ability to increase antioxidant defence system during toxicity (Ola-Mudathir et al, 2008).

The present study has demonstrated that administration of onion extract to rat may effectively prevent cyanide-induced nephro-toxicity in rat. This

effect is associated with increase in the activity of SOD and catalase, GST, and in the level of non-enzymatic antioxidant GSH in the kidney of the animals. Thus the antioxidant effect of this flavonoid and sulphur rich extract from *Allium Cepa* Linn is reflected in the present study. These results also suggest that onion extract may be useful nutritional antioxidants and its supplementation ameliorate the tissue damage or oxidative stress caused by cyanide. This effect is dose-dependent and is more pronounced than that of sodium thiosulphate.

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