

Assessing the efficacy of aqueous garlic extract, sodium nitrite and sodium thiosulfate against prolonged oral cyanide exposure in rabbits

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Abstract: This study was aimed to compare the efficacy of aqueous garlic extract, sodium nitrite (SNT), sodium thiosulfate (STS) and hydroxocobalamin against oral cyanide exposure in rabbits. For this purpose, forty two adult male rabbits were divided randomly into 7 groups of 6 animals (A-G) each. Rabbits in group A were offered feed only and served as negative control, while the rabbits in group B received feed plus potassium cyanide (KCN) at 3mg/kg orally and were kept as positive control. Animals in group C received feed, KCN and intraperitoneal injection (IP) of aqueous garlic extract at 500mg/kg. Rabbits in group D were given feed, KCN and IP injection of STS at 600mg/kg. Members in group E received feed, KCN and IP injection of both aqueous garlic extract at 500mg/kg and SNT at 20mg/kg. Animals in group F were given feed, KCN and IP injection of both STS at 600mg/kg and SNT at 20mg/kg, while the rabbits in group G received feed, KCN and IP injection of hydroxocobalamin at 300mg/kg. The treatments were given to respective groups for 40 days. The efficacy of the antidotes was measured on the basis of changes in biochemical profile of rabbits in each group. In this study, hydroxocobalamin was found to be significantly more effective cyanide (CNI) antidote than garlic, STS, SNT plus garlic extract, or SNT and STS, either alone or in combination. A combination of SNT and garlic extract was the second most effective CNI antidote. The efficacy of garlic alone was significantly higher than STS alone or in combination with SNT. The efficacy of combined SNT and STS was superior to STS alone in treating rabbits with CNI toxicity. In conclusion, aqueous garlic extract alone or in combination with STS can effectively be used against cyanide toxicity.

Keywords: Aqueous garlic extract, cyanide, rabbit, hydroxocobalamin, toxicity.

INTRODUCTION

Cyanide (CNI) is widely distributed in the ecosystem and has been linked to central nervous system (CNS) syndromes and thyroid in animals as well as humans (Soto-Blanco *et al.* 2005). Sorghum grazed cattle, horses and sheep exhibited ataxia and also urinary incontinence was observed in horses (McKenzie and Mc Micking, 1977; Bradley *et al.* 1995). Prolonged cyanide exposure has also been associated with reduced growth rate in animals (Tewe *et al.* 1984; Okolie and Osagie, 1999; Soto-Blanco *et al.* 2001), disturbance in thyroid metabolism (Philbrick *et al.* 1979; Okolie and Osagie, 1999), lesions in liver, kidneys, lungs (Okolie and Osagie, 1999, 2000) and also CNS pathology (Soto-Blanco *et al.* 2002a; Soto-Blanco *et al.* 2002b).

The onset of CNI toxicity is sudden. Thus, it necessitates a dynamic and immediate treatment to overcome the toxic effects. In order to get improved safety, a series of newer antidotes either alone or in adjunction with the conventional treatments have been examined (Way, 1983; Way *et al.* 1984; Isom and Borowitz, 1995; Crankshaw, *et al.* 2007; Borron *et al.* 2007; Oyewole and Olayinka,

2009). There is a wide variety of compounds that are used as CNI antidotes and on the basis of their mechanism of action are classified into four major groups. First scavengers, e.g. Sodium nitrite, amyl nitrate, hydroxocobalamin etc: are substances that inactivate CNI by binding it or by forming methaemoglobin, which in turn sequesters CNI. Second, detoxifying antidotes are compounds that detoxify CNI by enzymatic hydrolysis and convert it to a comparatively non-hazardous product readily excreted from the body e.g. Sodium thiosulfate, derivatives of mercaptopyruvate (Nagasawa, *et al.* 2007). Third, physiological antidotes: Such as oxygen and fourth, biochemical antidotes which have mainly unexplained mechanism of action and considered as non specific antidotes i.e. chlorpromazine (Isom and Borowitz, 1995; Bhattacharya *et al.* 1995).

A retrospective assessment of different antidotes against CNI indicates that there is no consensus about the effectiveness of a particular treatment protocol. This lack of consensus is mostly due to difference in experimental situations, test protocols and animals species employed in studying the efficacy of different antidotes. Implementation of a particular treatment in a country is also dictated by factors including government regulatory bodies and the legislations. Lacks of global unanimity

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also complicate the issue of antidote choice (Bhattarcharya. 2000). This paper describes the comparative evaluation of aqueous garlic extract, sodium nitrite, sodium thiosulfate and hydroxocobalamin against prolonged oral cyanide exposure in rabbits.

MATERIALS AND METHODS

Cyanide antidote trials were carried out in a rabbit model. For this purpose forty two adult rabbits of approximately same age and body weight supplied by University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore were used. The rabbits were housed individually in clean metal cages and were dewormed with Oxfendazole (Oxifax, ICI, Pvt., Pakistan). They were allowed to feed for two weeks before the start of the experiment for acclimatization. Afterwards, rabbits were divided randomly into 7 groups of 6 animals each viz. A-G. The experiment was carried out at 27°C (room temperature) and 12 hours day light cycle. Rabbits of group A were offered feed only and served as negative control, while the rabbits of group B received feed plus potassium cyanide (KCN) at 3mg/kg body weight (BW) orally and were kept as positive control. Animals in group C received feed, KCN at 3mg/kg BW orally and intraperitoneal injection (IP) of garlic extract at 500 mg/kg BW. Rabbits in group D were given feed, KCN at 3mg/kg BW orally and IP injection of STS 20% solution at 600mg/kg BW. Members in group E received feed, KCN at 3mg/kg BW orally and IP injection of both garlic extract at 500mg/kg BW and SNT 10% solution at 20 mg/kg BW. Animals in group F were treated with feed, KCN at 3mg/Kg BW orally and IP injection of both STS 20% solution at 600mg/kg BW and SNT 10% solution at 20mg/kg BW, while the rabbits in group G received feed, KCN at 3mg/kg BW orally and IP injection of hydroxocobalamin at 300mg/kg BW. The treatments were given to respective groups for a period of 40 days. Animal care and the experimental protocol applied were approved by the Ethical Committee of the UVAS, Lahore. Typical rabbit diet was prepared as per formula described by Singh (2005) with slight modification by replacing groundnut cake with cotton seed meal. Feed was given at 90g/kg/day. Fresh drinking water was provided *ad lib* while decayed feed leftovers were regularly removed and discarded.

Preparation of garlic extract

Garlic was purchased from the local market. Thirty grams (30gm) of peeled garlic was cut into small pieces and then crushed and homogenized with 60mL distilled water in a mortar and pestle. The crushed and homogenized garlic was decanted carefully to get 60mL of aqueous garlic extract. One milliliter of this aqueous extract contained 500 mg of garlic material (Elsaid and Elkomy, 2006). The garlic extract was prepared in biosafety cabin using sterilized both distilled water and mortar and pastel. After preparation the garlic extract was poured into sterilized

brown glass vials. The garlic extract was prepared fresh daily. The efficacy of the antidotes was measured on the basis of changes in biochemical profile.

Determination of biochemical parameters

At the end of 40 days, blood sample was collected from the ear vein of each rabbit using sterile disposable, 24 gauge needles and was drawn into clean, dry centrifuge tubes,. Freshly voided urine samples were obtained from the pots placed beneath each rabbit cage. After clotting blood samples were centrifuged for serum collection. The serum and urine were stored at 4°C and analyzed within 48 hours (Okolie and Osagie, 1999). The biochemical parameters like lactate dehydrogenase, alanine aminotransferase, aspartate transaminase, alkaline phosphatase, cholesterol, total protein, albumin, urea, creatinine, bilirubin, uric acid, urinary thiocyanate, and blood glucose were measured using standard procedures.

Postmortem examination of the rabbits

At the end of day 40, three rabbits from each group were euthanized by cervical dislocation to perform postmortem examination. Liver, kidneys, intestine, stomach, lungs, heart, brain and other tissues were observed for any gross changes.

STATISTICAL ANALYSIS

The data originating from the experiment were analyzed using two-way ANOVA and means of different groups were compared by least significant difference (LSD) test at a probability (*P*) level ≤ 0.5. A statistical software package “SPSS13.00” was used for statistical analysis.

RESULTS

The alanine transaminase (ALT) (EC 2.6.1.2) and aspartate transaminase (AST) (EC 2.6.1.1) values of rabbits in different treatment groups are shown in Table 1.

Table 1: Alanine transaminase (ALT) and aspartate transaminase (AST) values (U/L) as affected by CN⁻ in rabbits of various groups[†] A = Control, B = cyanide, C = garlic extract, D = sodium thiosulfate, E = sodium nitrite plus garlic extract, F = sodium nitrite plus sodium thiosulfate, G = hydroxocobalamin

Groups	Parameter	
	ALT (U/L)	AST (U/L)
A (n = 6)	43.33±1.282 ^a	50.16±1.195 ^a
B (n = 6)	78.33±1.256 ^b	93.5±1.118 ^b
C (n = 6)	54.66±0.954 ^c	60.5±0.885 ^c
D (n = 6)	64.50±0.991 ^d	73.33±2.231 ^d
E (n = 6)	50.83±0.600 ^e	56.00±1.032 ^e
F (n = 6)	61.33±0.955 ^f	65.16±1.276 ^f
G (n = 6)	44.66±1.022 ^{ag}	51.33±1.054 ^{ag}

[†]Values are mean ±SE, n = number of rabbits used, Means in the same column bearing different superscript letters (a, b, c, d, e, f, g) are statistically significantly different (*P*<0.05)

The ALT values were significantly higher ($P<0.05$) in groups B, C, D, E and F than control group A, while rabbits in group G were not significantly different from group A ($P>0.05$). Group B, ALT values were significantly increased ($P<0.05$) over those of groups C, D, E, F and G. The values of groups D and F were also significantly higher ($P<0.05$) than those of groups C and E. Groups D and F values were significantly different ($P<0.05$) as were group C and E. In contrast, group G has significantly lower ($P<0.05$) ALT values than groups C, D, E and F.

On the other hand, AST values of rabbits in groups B, C, D, E, and F were significantly increased ($P<0.05$) over control group A, whereas there was no difference between groups A and G ($P>0.05$). The rabbits of groups D and F showed significantly elevated ($P<0.05$) AST values compared to groups C, E and G. Also significant difference ($P<0.05$) was found in AST values between groups D and F. Similarly the difference between groups C and E was also significant ($P<0.05$). The AST values of rabbits in group G were significantly lower than those of groups C, D, E and F.

The values of alkaline phosphatase (ALP) (EC 3.1.3.1) and lactate dehydrogenase (LDH) (EC 1.1.1.27) enzymes are depicted in table 2.

Table 2: Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) values of rabbits in different treatment groups[†] A = Control, B = cyanide, C = garlic extract, D = sodium thiosulfate, E = sodium nitrite plus garlic extract, F = sodium nitrite plus sodium thiosulfate, G = hydroxocobalamin

Groups	Parameter	
	ALP (U/L)	LDH (U/L)
A (n = 6)	5.33±0.422 ^a	42.33±0.989 ^a
B (n = 6)	17.67±0.715 ^b	82.50±0.719 ^b
C (n = 6)	10.17±0.946 ^c	58.83±1.352 ^c
D (n = 6)	14.67±0.667 ^d	71.16±1.662 ^d
E (n = 6)	8.00±0.365 ^e	52.16±1.537 ^e
F (n = 6)	12.50±0.764 ^f	64.16±0.703 ^f
G (n = 6)	5.83±0.401 ^{ag}	44.33±0.918 ^{ag}

[†]Values are mean ±SE, n = number of rabbits used, Means in the same column bearing different superscript letters (a, b, c, d, e, f, g) are statistically significantly different ($P<0.05$)

The ALP values of rabbits in group G were not significantly different ($P>0.05$) from control group A. The ALP values of rabbits in group G were significantly lower compared to groups C, D, E and F. While rabbits in groups C, D, E and F showed significantly decreased ALP values ($P<0.05$) compared to those in control B. Alkaline phosphatase values of groups D and F were significantly higher compared to groups C and E. The rabbits of group D showed significantly higher ALP values than group F.

Similarly significantly higher ($P<0.05$) ALP values were observed in group C over group E.

Likewise, mean LDH values of rabbits observed were 42.33±0.989, 82.50±0.719, 58.83±1.352, 71.16±1.662, 52.16±1.537, 64.16±0.703 and 44.33±0.918 U/L in groups A, B, C, D, E, F and G, respectively. The LDH values of rabbits in groups B, C, D, E and F were observed significantly higher ($P<0.05$) than control group A. While the LDH values of group G were non-significant ($P>0.05$) with control group A. When compared the LDH values of groups C, D, E and F with group B the difference was highly significant ($P<0.05$). Lactate dehydrogenase levels of groups D and F was increased significantly compared to rabbits in groups C and E. Rabbits in group D showed significantly increased ($P<0.05$) LDH level than group F. The LDH difference between groups C and E was also observed significant ($P<0.05$). The rabbits in group G demonstrated significantly decreased LDH levels than treatment groups C, D, E and F.

The effect of treatment on serum total protein, albumin and bilirubin levels of rabbits is shown in table 3.

The serum albumin levels in rabbits of groups B, C, D, E and F were significantly decreased ($P<0.05$) compared to control group A, while the difference between serum albumin levels for groups A and G was not significant ($P>0.05$). Rabbit albumin values in groups C, D, E, F and G were significantly higher ($P<0.05$) than those of group B. When compared to albumin values of group D and F with those of group C and E the difference was significant ($P<0.05$). Albumin serum values of group D were significantly lower ($P<0.05$) than group F, as were also those of group C ($P<0.05$) when compared with albumin values of group E. The serum albumin levels of rabbits in group G were significantly higher than any of the groups C, D, E and F.

Similar pattern of significance in serum TP was observed in serum albumin. Total serum proteins levels were significantly lower ($P<0.05$) in groups B, C, D, E and F compared to control group A. The serum TP levels of group G was not significantly different ($P>0.05$) than control group A. Serum protein levels of rabbits in group B, were significantly reduced ($P<0.05$) below either treatment group C, D, E, F and G. Group G showed significantly higher ($P<0.05$) serum proteins values than either of the treatment groups C, D, E or F. Group C demonstrated significantly lower level of serum proteins than group F. Similarly rabbits in group C exhibited significantly higher ($P>0.05$) serum proteins values than group E, whereas those of both the groups D and F showed significantly higher ($P<0.05$) serum protein values than group C or E.

Table 3: Albumin, Total protein and bilirubin values of rabbits in various groups[†] A = Control, B = CNI, C = garlic extract, D = sodium thiosulfate, E = sodium nitrite plus garlic extract, F = sodium nitrite plus sodium thiosulfate, G = hydroxocobalamin

Groups	Parameter		
	Albumin (gm/dl)	Total Proteins (gm/dl)	Bilirubin (mg/dl)
A (n = 6)	3.53±0.242 ^a	7.01±0.102 ^a	0.20±0.026 ^a
B (n = 6)	1.18±0.070 ^b	1.89±0.102 ^b	1.42±0.070 ^b
C (n = 6)	2.47±0.105 ^c	5.15±0.799 ^c	0.65±0.043 ^c
D (n = 6)	1.62±0.108 ^d	3.03±0.087 ^d	0.97±0.041 ^d
E (n = 6)	3.07±0.157 ^e	6.14±0.119 ^e	0.45±0.022 ^e
F (n = 6)	2.07±0.061 ^f	4.13±0.121 ^f	0.83±0.033 ^f
G (n = 6)	3.42±0.179 ^{ag}	6.51±0.087 ^{ag}	0.32±0.031 ^{ag}

Table 4: Serum uric acid, urea and creatinine values of rabbits in various groups[†] A = Control, B = cyanide, C = garlic extract, D = sodium thiosulfate, E = sodium nitrite plus garlic extract, F = sodium nitrite plus sodium thiosulfate, G = hydroxocobalamin

Groups	Parameter		
	Uric Acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
A (n = 6)	1.166±0.033 ^a	9.33±0.882 ^a	0.50±0.068 ^a
B (n = 6)	4.016±0.160 ^b	22.83±0.131 ^b	1.98±0.075 ^b
C (n = 6)	2.300±0.093 ^c	13.43±0.289 ^c	1.12±0.095 ^c
D (n = 6)	3.018±0.045 ^d	20.72±0.189 ^d	1.72±0.117 ^d
E (n = 6)	1.716±0.090 ^e	11.74±1.040 ^e	0.82±0.048 ^e
F (n = 6)	2.750±0.056 ^f	15.32±0.313 ^f	1.40±0.089 ^f
G (n = 6)	1.250±0.084 ^{ag}	9.45±0.182 ^{ag}	0.58±0.070 ^{ag}

[†]Values are mean ±SE, n = number of rabbits used, Means in the same column bearing different superscript letters (a, b, c, d, e, f, g) are statistically significantly different ($P < 0.05$)

Serum bilirubin level of rabbits in groups B, C, D, E and F were significantly increased ($P < 0.05$) above control group A. While the difference between groups G and control group A was not significant ($P > 0.05$). The rabbits in groups C, D, E and F exhibited significantly reduced ($P < 0.05$) serum bilirubin levels than group B. On the other hand the rabbits in group G showed significantly lower ($P < 0.05$) serum bilirubin values than either of the groups C, D, E and F. Groups D and F demonstrated significantly higher ($P < 0.05$) serum albumin concentrations than either of the treatment group C or group E rabbits. The difference between groups D and F was significant. Likewise the difference in serum bilirubin concentration between groups C and E rabbits was also significant ($P < 0.05$).

The uric acid, urea and creatinine concentrations in serum of rabbits in different groups are shown in table 4. Serum uric acid levels of groups B, C, D, E and F were significantly higher ($P < 0.05$) than control group A. The difference was non-significant ($P > 0.05$) in uric acid concentrations between rabbits of group G and control group A. The rabbits of treatment group G represented significantly lower ($P < 0.05$) uric acids concentration than groups C, D, E and F. Serum uric acid levels of rabbits in group B were significantly elevated ($P < 0.05$) over groups C, D, E and F. Groups D and F exhibited significantly

higher ($P < 0.05$) concentration of serum uric acid than groups C and E. Serum uric acid levels were significantly higher ($P < 0.05$) for Group D than group F. Likewise rabbits of group C demonstrated significantly higher ($P < 0.05$) uric acid concentrations than group E.

The difference in urea levels between group G and control group A was not significant ($P > 0.05$). Groups B, C, D, E and F had significantly higher serum urea levels than control group A ($P < 0.05$). The groups C, D, E and F exhibited a significant increase in serum urea levels ($P < 0.05$) than group G. Whereas the values of serum urea of group B were significantly higher ($P < 0.05$) compared to groups C, D, E, F and G. Serum urea levels of group D and F significantly increased over groups C and E. Serum urea levels of group D was significantly higher ($P < 0.05$) when compared to group F.

Serum creatinine levels for group B were significantly higher ($P < 0.05$) than control group A as well as groups C, D, E, F and G. While serum creatinine values for groups C, D, E and F were significantly increased ($P < 0.05$) above control group A. The differences in serum creatinine values were not significant ($P > 0.05$) between groups A and G. In addition, group G exhibited significantly lower ($P < 0.05$) serum creatinine levels than rabbits of either groups C, D, E, and F. Those in group D showed

significantly higher ($P < 0.05$) serum creatinine values compared to group F. Also, the levels of creatinine in group C were significantly elevated ($P < 0.05$) over group E.

The serum concentrations of rabbit triiodothyronine (T3) and thyroxine (T4) hormone in various groups are shown in Table 5. It was observed that the T3 levels in group B were significantly lower ($P < 0.05$) compared to control group A. Similarly, the T3 values of groups C, D, E and F were also significantly decreased ($P < 0.05$) compared to control group A. The difference of T3 levels was not significant between treatment group G and control group A. While the T3 concentrations of groups C, D, E and F were significantly less ($P < 0.05$) compared to group G. Groups D, F and C had significantly less ($P < 0.05$) T3 levels than group E. The difference was not significant between group C and F ($P > 0.05$). While the difference in T3 levels of group C rabbits was significantly decreased ($P < 0.05$) compared to group E.

The T4 levels in group B were significantly lower ($P < 0.05$) compared to control group A. Similarly, the T4 values of groups C, D, E and F were also significantly decreased ($P < 0.05$) compared to control group A. The difference of T4 levels was not significant between treatment group G and control group A. While the T4 concentrations of groups C, D, E and F were significantly less ($P < 0.05$) compared to group G. Groups D, F and C had significantly less ($P < 0.05$) T3 levels than group E. The difference was not significant between group C and F ($P > 0.05$). While the difference in T4 levels of group C rabbits was significantly decreased ($P < 0.05$) compared to group E.

Blood glucose, serum cholesterol and urinary thiocyanate (SCN) levels of rabbits in different groups are shown in Table 6. No significant difference ($P > 0.05$) in blood glucose levels of rabbits in groups A, B, C, D, E, F and G was observed. This indicated that CN^- did not induce any harmful effect on glucose tolerance of rabbits. Similarly the difference of serum cholesterol values among rabbits of different treatment group and control groups were not significant ($P > 0.05$). Urinary SCN levels of groups B, C, D, E, F and G was significantly higher ($P < 0.05$) compared to control group A. No significant difference ($P > 0.05$) was found in SCN levels of group C and E. Similarly, SCN levels of group D were non-significant ($P > 0.05$) to group F. Urinary SCN levels of groups C and E were significantly higher ($P < 0.05$) compared to groups D and F.

Postmortem examination

None of the treatment groups demonstrated any of the gross changes in any organ. Liver was normal in size, shape, texture and color. Kidneys were also normal in size and color. Lungs and brain were also normal in color and

consistency. Heart, intestine, pancreas and other organs also showed normal gross structures.

DISCUSSION

To better address CNI toxicity, a number of new antidotes either singly or in combination with formerly used treatments have been evaluated (Crankshaw *et al.* 2007; Oyewole and Olayinka, 2009; Bebarta *et al.* 2016). Cyanide antidotes have been grouped into four classes, based on the mechanism of detoxification. These included scavenging, detoxifying, physiological, and biochemical (Isom and Borowitz, 1995).

In the present study, hydroxocobalamin was found to be a significantly more effective CNI antidote than garlic, sodium thiosulfate (STS), sodium nitrite (SNT) plus garlic extract, or SNT and STS, either alone or in combination. A combination of SNT and garlic extract was the second most effective CNI antidote. The efficacy of garlic alone was significantly higher than STS alone or in combination with SNT. The efficacy of combined SNT and STS was superior to STS alone in treating rabbits with CNI toxicity. Sodium thiosulfate uses the rhodanese enzyme pathway to detoxify CNI from the blood (Hall and Rumack, 1986). The underlying reason for the lower efficacy of STS may be the decreased availability of rhodanese in the extra cellular space, where CNI toxicity occurs (Sylvester *et al.*, 1983). According to Westley (1980), rhodanese has enhanced activity in mitochondria, while STS has low intracellular distribution (Way *et al.*, 1984). In addition, according to Way *et al.* (1984), in mammals, abundant amounts of rhodanese are present in liver, while CNI predominantly affects heart and brain tissue, and STS has limited distribution in these tissues (Baskin *et al.*, 1992). In most animal studies, STS alone has been compared to its combination with other compounds such as SNT and hydroxocobalamin. According to Hug and Marenzi (1933), the addition of either methylene blue or SNT created a more efficacious treatment in dogs than STS alone. In a study of rabbits administered CNI by gavage, Hug and Marenzi (1933) reported that seven of ten animals died following exposure, despite IV administration of STS. They also observed that STS, normally nontoxic to healthy animals, led to shock and reduced the time to cardiac arrest and death in CNI exposed animals. Similar effects were observed when STS and SNT were used in combination (Hug and Marenzi, 1933). Sodium thiosulfate requires a latent phase to exert its antidotal action, and its effects against CNI was more likely prophylactic rather than curative (Hug 1932). Sodium thiosulfate has been shown more effective in the initial phase of apnea than later in the course of toxicity. Sodium nitrite combined with STS, hydroxocobalamin with STS, 4-DMAP, and dicobalt-EDTA were more efficacious during later phases of toxicity (Paulet and Dassonville, 1985). Renard *et al.*

(2005) administered KCN to rats followed by IP STS injection at the time when CNI in blood was assumed to peak and found that rats exhibited significantly lower arterial blood CNI levels, arterial and venous lactate levels, and venous PO₂s, indicating curative efficacy for these CNI toxicity markers (Renard *et al.*, 2005). Sodium nitrite and STS, conventional antidotes to CNI, should not be used as prophylaxis due to their side effects (Baskin *et al.* 1992). Furthermore, the short biological half life of STS and small distribution volume limit its utility as CNI antidote (Baskin *et al.*, 1999).

Garlic is well known for its medicinal uses and its possible ability to reduce the risk of some diseases, but no reports in the literature are available on its curative or protective effects against CNI poisoning (Aslani *et al.*, 2006). Various sulfur compounds are present in garlic at concentrations higher than in other species of Allium (Newall *et al.*, 1996). About 1% alliin (S-allyl cysteine sulfoxide) is present in dried garlic powder. The active compound of garlic is allicin (diallylthiosulfinate) which is not produced until injury to the garlic bulb. Damage to garlic bulbs yields allinase, an enzyme responsible for metabolizing alliin to allicin (Block. 1996). The precise protective effect of garlic against CNI toxicity has not been established, but with respect to the metabolic pathways of CNI, protection against mortality afforded by garlic may be due to its sulfur compounds (Aslani *et al.*, 2006). In the present study, garlic extract was found to be more effective than either STS alone or in combination with SNT. These findings were similar to the results of Elsaid and Elkomy (2006) who found garlic extract was more effective than STS alone and also more effective than a mixture of garlic extract and STS.

Cyanide toxicity is also caused by increased generation of super oxide anions and lipid per oxidation with inhibition of antioxidant enzymes (Ardelt *et al.*, 1994). Elsaid and Elkomy (2006) found CNI intoxication to be associated with increased malondialdehyde (MDA) levels and with decreased catalase and super oxide dismutase (SOD) activity in liver and kidney, indicating toxicity to these tissues. Garlic extract scavenges hydroxyl radicals and superoxide anions, hence modulating lipid per oxidation (Kim *et al.* 2001; Saravanan and Prakash, 2004) that is the possible reason for reduction in MDA levels, catalase, and SOD activity in liver and kidney tissues after garlic extract administration (Elsaid and Elkomy, 2006). Moreover, due to oxidative stress induced by CNI, the activity of glutathione (GSH), glutathione reductase (GSR), and glutathione-s-transferase (GST) become reduced, but were restored to normal values following garlic treatment (Elsaid and Elkomy, 2006). Banerjee *et al.* (2002) suggested this restoration to be related to antioxidant activity of garlic extract. Sulfur is required for the production of GSH (Kim and Kim, 1999; Wu *et al.*,

2001), and diallyl disulfide plus diallylsulfide (garlic components) may be the source of required sulfur (Elsaid and Elkomy, 2006). Consequently GSH level was restored, resulting in increase in the activity of GSR and GST (Saravanan and Prakash, 2004). Organo-sulfur compounds present in garlic oil enhanced the generation of GST (Kim *et al.* 1996) as well as cellular GSH in erythrocytes (Wu *et al.* 2001). Due to its detoxification properties, GST catalyzed the binding of many electrophilic agents with GSH (Hayes and Pulford, 1995). Hence it may bind to CNI, explaining the decrease of GST and GSH in both liver and kidneys (Elsaid and Elkomy, 2006). The organo-sulfur component (as diallyl sulfide) in garlic oil also shows hepato-protective effects against toxins (Kwak *et al.* 1994). Restoration of ALT, AST, and bilirubin as well as albumin in garlic treated rabbits to near control values in this study may be attributed to this hepato-protective effect of garlic.

Elevated levels of serum uric acid, creatinine and urea found in this study indicated renal toxicity. These levels were restored to near control values in garlic treated rabbits, which may be due to antioxidant properties of the garlic (Elsaid and Elkomy, 2006). In the present study, CNI also significantly decreased T3 and T4 levels, reflecting thyroid damage; values were restored to near normal using garlic extract, in agreement with Elsaid and Elkomy (2006). Garlic extract has been reported to restore thyroid hormone levels to near control values (Elsaid and Elkomy, 2006), since it can induce the formation of GSH (Geng and Lau, 1997), important in detoxifying CNI, by reducing the free SCN level in plasma and making iodine available for thyroid function.

In the present study, hydroxocobalamin was found to be the most effective CNI antidote over SNT, STS, garlic extract, or their combinations. The use of hydroxocobalamin as a CNI treatment was first established in 1952 (Mushett *et al.*, 1952). The detoxification pathway involves directly chelating CNI to make cyanocobalamin, which is non-toxic and is passed out in the urine. An *in vitro* study revealed that addition of hydroxocobalamin to human fibroblasts incubated in a CNI solution reduced intracellular CNI by 75% and resulted in the formation of intracellular cyanocobalamin, which reflected cellular penetration and the action of hydroxocobalamin (Astier and Baud, 1996). Experiments conducted in animals have revealed that hydroxocobalamin can cross the blood-brain barrier, hence entering the cerebrospinal fluid (Van den Berg *et al.*, 2003). Hydroxocobalamin has also been tested as a CNI antidote in various animal models including rabbits (Vincent *et al.*, 1981), guinea pigs (Posner *et al.*, 1976), mice (Crankshaw *et al.* 2007), baboons (Posner *et al.* 1976), dogs (Borron *et al.*, 2006), and rats (Oyewole and Olayinka, 2009). In an evaluation of hydroxocobalamin efficacy for the treatment of KCN exposed dogs (Paulet

and Olivier, 1963), mortality rate was higher (82%) in a saline control group, with death in 4 hours to 4 days. Survival rate in the hydroxocobalamin groups, treated at 75 or 150 mg/kg, was 79% and 100%, respectively. These animals survived longer than 14 days post-poisoning and exhibited no neurological sequelae. Treatment with hydroxocobalamin leads to increase in blood pressure beginning 1 to 3 minutes after start of infusion, which is beneficial (Paulet and Olivier, 1963). According to Oyewole and Olayinka (2009) rats receiving amygdaline died of CNI poisoning, while no mortality was recorded in hydroxocobalamin treated rats. Blood CNI and lactate levels were significantly reduced in hydroxocobalamin treated rats compared to controls. Hydroxocobalamin also reversed increased PCV, hemoglobin, and pH to normal values in rats, and no alterations in tissue architecture were found in hydroxocobalamin treated rats (Oyewole and Olayinka, 2009), which is congruent with the results of this study. In humans exposed to acute CNI poisoning, 71% survival was recorded after treatment with hydroxocobalamin, which is considered as first line treatment in acute CNI toxicity cases (Borron *et al.*, 2007). Hydroxocobalamin also has an improved safety profile for children, pregnant women, and smoke inhalation victims. Therefore hydroxocobalamin could be a superior choice in uncertain CNI poisoning cases (Shepperd and Velez, 2008).

No rabbits in any group demonstrated gross deviation from the normal organ structure. Liver, kidney, lung, and brain all showed size, shape, texture and color within normal limits. Heart, intestine, pancreas and other organs also showed normal gross structure. These findings are consistent with those of Soto-Blanco *et al.* (2005) who found no gross lesions in any organ in necropsied goats after exposure to CNI. The results of present study were also in agreement with those of Odriozola *et al.* (2009).

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