Toxicity Evaluation of *Cnidoscolus Aconitifolius* on Female Albino Wistar Rats

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ABSTRACT: Toxicity evaluation of Cnidoscolus aconitifolius on female albino Wistar rats was investigated by monitoring toxicity indices such as serum glutathione, antioxidant capacity and elevated cyanide in serum and urine and elevated serum thiocyanate and nitrite. Feeding of female albino Wistar rats with Cnidoscolus aconitifolius containing 12.7 mg CN-1kg-1wt/wt gave the following results; group D (animals fed with 50% Cnidoscolus leaves) had the highest mean concentration value (538.19 ± 39.28) for total cyanide (TCN)(µg/ml) in urine while the control group (animals fed with only commercial feed) had the least concentration value(29.65 ± 4.42) in urine and for free cyanide (FCN)(µg/ml) in urine, group D had 53.39 ± 1.66 while group A had 0.7 ± 0.68. But for serum total cyanide (µg/ml), the control group had the highest mean concentration value of 3.079 ± 1.52 and 1.266 ± 0.1 for serum free cyanide (FCN)(µg/ml) whereas group D had 0.358 ± 0.102 for serum total cyanide and 0.106 ± 0.08 for serum free cyanide. Total antioxidant capacity of the analysis was also observed using Sorbo method (1953) with respect to thiocyanate ion concentration in serum (µm/ml). Serum thiocyanate concentration was found to occur highest in group D with 3.50 ± 0.27 and the control group had the least with 0.15 ± 0.03. Decrease in whole blood glutathione (an important biological antioxidant) was also observed and group D contained the least mean value with -0.018 ± 0.008 (µg/ml) while the control group had the highest with 0.260 ± 0.003 (µg/ml). Increase in nitrite (N-nitrosamine precursor) was also observed. Group D had 19.94 ± 2.00 mean concentration value (µm/ml) whereas the control group had the least with 9.62 ± 1.17 (µm/ml). The toxicological implications of these findings are discussed.

Key words: Albino Wistar rats, Cnidoscolus aconitifolius, Cyanide, Glutathione, Serum, Toxicity, Urine

INTRODUCTION

Cnidoscolus aconitifolius is popularly called tree spinach.

It is a large fast growing green leafy perennial shrub, native to the Yucatan peninsula of Mexico but is now found almost in all parts of the world including the sub-Saharan part of Africa and Southern Nigeria in particular. It is consumed mostly by people of the southern part of Nigeria may be due to its anemia-ameliorating and blood boosting capacity. Women however, are known to consume more of this plant especially those that gave birth newly or in anemic conditions so as to help improve on their blood level. But the danger remains that they feel that consuming it raw tends to give a greater blood boosting impact than when consumed cooked. This plant has been found to contain substantial amount of cyanide and some people tend to eat it raw which is an unwise thing to do [14].

Toxicity evaluation of this plant however, cannot be observed or investigated in these women, hence the use of female albino Wistar rats for the investigation.

This plant is taxonomically recognized as belonging to *Cnidoscolus aconitifolius* [10]. The name *Cnidoscolus chayamansa* by McVaugh is still sometimes used [16]. Ross-Ibarra and Molina-Cruze recognized four varieties that are cultivated and are morphologically distinct [20].

It is an evergreen plant of 20 feet high, with short stout trunk of 6 inches in diameter. Produces white flowers when mature and have leaves that contain scattered stinging hairs all over their body. It is usually used for ornamental purposes and serves as shed tree along the city streets and near houses in most part of Puerto Rico. It is usually propagated from cuttings and germinates very easily.

Chayamansa leaves have been found to be of nutritive value with protein content (5.7%), crudefibre (1.9%), calcium (199.4mg/100g), potassium (217.2mg/100g), iron (911.4mg/100g), vitamin C (164.7mg/100g) and carotene (0.085mg/100g). The leaves of *Chaya* leaf nutrients above agree with published reports of Booth *et al*., 1992 [3] and are two to three folds greater than most edible leafy green vegetables.

This plant is drought resistant which is of a particular value to areas that experience intermittent rainfall.

MATERIAL AND METHOD

Twenty female albino Wistar rats weighing between 130-184g and of 7 to 8 weeks old were purposively selected for the investigation. The rats were purchased from a local animal husbandry in Nsukka, Enugu State in South-Eastern Nigeria. The animals were acclimatized to their environment and fed with commercial rat feed for one week before feeding with treatment feed commenced. They were housed in spacious rubber cages surrounded with metal gauze (five animals per cage) and maintained in a well-ventilated room with temperature of about 20-220C. They received pellet diets and water given ad libitum. By random selection, they were divided into four groups. One control group (group A) and three test groups (i.e., group B, C and D), five animals belonging to each group.

The control group was fed with commercial feed only while the test groups were fed with treatment feeds. 20g of the feed were about the quantity eachrat could consume between 12 to 48hours of feeding. At the 8th day of feeding with test feeds, portions of 0.3ml of NaNO2 solution was administered to each animal in each group by stomach tube. Before decapitation of the rats, blood was collected directly from the heart through cardiac puncture with a syringe. This immediately follows the test for whole blood glutathione (GSH) level of each animal from each group, using Ellman's method (1959) [5].

4ml of blood were gotten from each rat. The remaining blood after that of GSH test was removed was centrifuged for 15mins at 2500 rev/min to get the serum for analysis. The serum of each rat was stored in the refrigerator at 2 to 40C. The livers, kidneys, small intestines, stomachs of the rats were removed and washed with normal saline and weighed. Urines were collected by puncturing the bladders of the rats with syringes after dissection.

Total cyanide of Cnidoscolus aconitifolius was determined according to Esser (1993) [7]. Cyanide in serum and urine was estimated using the method of Esser (1959) [6]. Nitrite determination of fresh leaves of Cnidoscolus aconitifolus was done using Follet and Ratcliff method (1963) [8]. Thiocyanate analysis in serum was done using Sorbo Method (1953) [22].

Treatment feed: The fresh Cnidoscolus aconitifolius (tree spinach) used in the experiment was harvested from a private garden at Umuosu Nsulu, Isiala Ngwa North Local Government Area of Abia State, Nigeria. The leaves were ground into semi-liquid paste using an electric homogenizer. The treatment feed was prepared by mixing 30%, 40%, and 50% of ground leaves of Cnidoscolus aconitifolius with 70%, 60%, and 50% respectively of commercial feed and pelleting the mixture and this was allowed to air-dry and put in different cellophane bags according to their respective percentages to avoid mix-ups.

The commercial rat pallets were purchased from a local market in Umuahia, Abia State.

Statistical Analysis:

Student's t-test was used to get the Least Significant Difference (LSD) of each treatment means and ANOVA tables were created and from where significant (s) and non-significant (ns) differences of the treatments are tabulated.

RESULTS

STANDARD CURVES FOR THE ANALYSIS

Table 1

POTASSIUM CYANIDE STANDARD CURVE

Vol. of stock (ml)	Vol. of diluent (ml)	Absorbance (nm)	Concentration (µg/ml)
0.0	1.0	0.000	0.000
0.1	0.9	0.011	0.129
0.2	0.8	0.014	0.165
0.3	0.7	0.026	0.306
0.4	0.6	0.039	0.459
0.5	0.5	0.042	0.494
0.6	0.4	0.051	0.576

Table 2

GLUTATHIONE (GSH) STANDARD CURVE

Vol. of stock (ml)	Vol. of diluent (ml)	Absorbance (nm)	Concentration (µg/ml)
0.00	0.50	0.00	0.048
0.01	0.49	0.05	0.001
0.02	0.48	0.07	0.020
0.03	0.47	0.08	0.030
0.04	0.46	0.09	0.040
0.05	0.45	0.11	0.060
0.06	0.44	0.13	0.079

Table 3

NITRITE (NO2) STANDARD CURVE

Vol. of stock (ml)	Vol. of diluent (ml)	Absorbance (nm)	Concentration (µg/ml)
0.0	1.0	0.000	-0.069
0.1	0.9	0.126	0.106
0.2	0.8	0.184	0.186
0.3	0.7	0.311	0.363
0.4	0.6	0.391	0.494
0.5	0.5	0.402	0.489
0.6	0.4	0.471	0.585

Table 4

THIOCYANATE STANDARD CURVE

Vol. of stock (ml)	Vol. of diluent (ml)	Absorbance (nm)	Concentration (µg/ml)
0.0	0.10	0.000	-0.061
0.1	0.90	0.035	0.015
0.2	0.80	0.062	0.071
0.3	0.70	0.092	0.139
0.4	0.60	0.101	0.159
0.5	0.50	0.220	0.417
0.6	0.40	0.308	0.609

MEAN WEIGHTS OF ORGANS

Table 5

MEAN WEIGHTS OF THE ORGANS OF THE CONTROL GROUP AND TEST GROUPS.

Organ	Group A (control)	Group B (30%)	Group C (40%)	Group D (50%)
Stomach	21.308	21.710	20.838	20.222
Small intestine	11.382	11.190	10.802	9.590
Liver	4.920	4.788	5.762	5.040
Kidney	1.034	1.002	1.016	0.828

CYANIDE IN SERUM AND URINE

Table 6

TOTAL CYANIDE (TCN) AND FREE CYANIDE (FCN) IN URINE AND SERUM OF RATS (µg/ml)

Group	Sample			
	Urine		Serum	
	TCN	FCN	TCN	FCN
A	29.65 ± 4.42	0.7 ± 0.68	3.079 ± 1.52	1.266 ± 0.1
B	221.70 ± 8.68	19.21 ± 5.51	2.544 ± 0.041	0.572 ± 0.28
C	289.84 ± 16.45	30.10 ± 2.35	2.012 ± 0.32	0.286 ± 0.16
D	538.19 ± 39.28	53.39 ± 1.66	0.358 ± 0.102	0.106 ± 0.08

The results of total cyanide and free cyanide in urine and serum are reported as means with their standard deviations (mean ± SD). The total cyanide (TCN) and free cyanide (FCN) of the urine of group D which was the group that consumed the highest proportion of the plant (50%Cnidoscolusaconitifolius) had the highest values when compared with the urine of other test groups and that of the control group that did not feed on the plant sample). Also, the serum of group D had the highest value of TCN and FCN when compared with other test groups and the control group. But the values of TCN and FCN obtained from urine were significantly greater than those of the serum.

Table 7

CONCENTRATION OF WHOLE BLOOD GLUTATHIONE (µg/ml)

A	B	C	D
0.260 ± 0.003	0.163 ± 0.003	0.119 ± 0.03	-0.018 ± 0.008

From the above table, whole blood GSH concentration was greater in the control group followed by group B and was negative in group D.

Table 8

NITRITE CONCENTRATION IN *CNIDOSCOLUS ACONITIFOLIUS* (µm/ml)

A	B	C	D	FRESH CNIDOSCOLUS ACONITIFOLIUS
9.62 ± 1.17	11.82 ± 3.48	12.25 ± 0.81	19.94 ± 2.00	0.049

From table 8 above, nitrite concentration in serum was greatest in group D followed by group C but least in group A. the value obtained from the plant was significantly smaller than those of the serum.

Table 9

THIOCYANATE ION CONCENTRATION IN SERUM (μm/ml)

A	B	C	D
0.15 ± 0.03	1.71 ± 0.21	2.61 ± 0.44	3.50 ± 0.27

From the values obtained in table 9 above, the concentration of thiocyanate ion in serum increased as proportions of plant samples increased and the animals in group A had the least concentration of thiocyanate ion. That is, groups D had the highest concentration of thiocyanate ion, followed by group C, B, and A in that order.

DISCUSSION

The mean total cyanide (TCN) and free cyanide (FCN) levels in urine of group D animals were significantly higher than that of the control group ($P < 0.05$). This could be as a result of exposure to Cnidoscolus cyanide arising from ingestion of Cnidoscolus leaves in test feed. The mean TCN and FCN level in serum at group D animals was significantly lower than that of the control group ($P < 0.05$). This suggests that there is more glutathione activity/depletion in rats' sera of the group D animals than that of the control group animals which depletes glutathione concentration, a process that donates electrons to free radicals in order to stop oxidative stress caused by oxidants from the plant.

Serum thiocyanate was significantly higher in group D than in group A. The high serum thiocyanate level in group D suggests that thiocyanate is an indication of the attempt of the animals to detoxify the ingested cyanide [4]. The sera of control group had the least thiocyanate value because there were little or no cyanide content in the commercial feed which they were fed with. One of theobviousimplications of exposure to cyanide is the depletion in the body's aminoacid pool, since sulfur-containing amino acids are needed for cyanide detoxification [2]. It has been well established that rhodanase enzyme and mercaptopyruvate sulfurtransferase enzyme utilize sulfur containing amino acids for cyanide detoxification [17]. Higher concentration of cyanide in urine above the corresponding thiocyanate in serum after the period of investigation clearly indicates low conversion of cyanide to thiocyanate. This resulted in accumulation of cyanide within the system. In this context, cyanide is known to be a free radical on some target organs [9].

The mean nitrite in serum of the group D had the highest significant difference ($P < 0.05$) and the control group had the least. This is as a result of 50% intake of Cnidoscolus leaves in test feed. Increased nitrite level indicates increased interaction with hemoglobin forming methemoglobin by oxidation of ferrous ion to ferric state preventing the blood to transport oxygen. This condition is well known as methemoglobinemia [19],[11]. Acute toxicity of nitrite could give rise to decreases such as oxygen deficiency, irradiation of the gastro-intestinal tract (GIT), and even sudden death while chronic toxicity could cause disturbances of fertility. Also, formation of N-nitrosamines produces carcinogenic effect on liver, oesophagus, respiratory system and kidney. They exert their adverse biological effect after being metabolically activated by microsomal mixed-function oxidases to form reactive intermediate [13]. There have been several studies done on the physiological and pathophysiological function of the nitrite in recent years. It forms peroxinitrite anion by reacting with superoxide radical [18].

Glutathione is a reducing agent that fights against reactive intermediate of metabolism, drugs and carcinogens [15].

In this study, mean GSH level in serum of group D animals was significantly lower than that of the control group ($P < 0.05$). This suggests that raw or uncooked Cnidoscolus leaves creates oxidative stress in the body of animals while glutathione donates electrons to quench the chain reaction set off by oxidants from the leaves, thus, depleting the level of glutathione in blood.

GSH determination in blood is well established as an accurate indicator of whole body GSH status. From this study, an implication of cyanide detoxification is decreased concentration of the glutathione in the body. This could be in part due to reduced synthesis of this important biological compound. In this connection, cysteine, a sulfur-containing amino acid which is needed for cyanide detoxification in the body [17] is the limiting amino acid in glutathione synthesis. Liu et al., 1999 [12] reported result on liver tissue levels of GSH activity which slightly relates to this study. The decrease in the GSH may be a substrate for glutathione peroxidase to protect increasing formation of reactive

oxygen species (ROS) [23]. However, some studies reported increase in the level of GSH in liver [1],[21].

CONCLUSION

The result of this study indicates the effects of Cnidoscolu saconitifolius leaves on antioxidant activity in the body of the animals and since there was a significant reduction in glutathione concentration in blood of the animals, it could be deduced that the condition is as a result of the free radicals produced from cyanide and nitrite intoxication. Therefore fresh Cnidoscolus aconitifolius should be precooked or cooked in order to remove excess cyanide and nitrite as well as preventing cyanide and nitrite intoxication.

RECOMMENDATION

The adult humans especially women with respect to the South-Eastern part of Nigeria who feel that the blood boosting potency of the plant is acquired more when the plant was homogenized, mashed and filtered and the fresh juice taken raw should be discouraged due to the dangers this could pose to their health. However, the best way to consume this plant is to parboil or pre-cooked or cook it in order to remove the cyanogenic components of it. This way, its presumed blood boosting capacity still remains intact.

REFERENCE

[1] Aykae, G., Uysal, M., Yalem, A. S., Kocak, N., Sivas, A. and Oz, A."The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione peroxidase and glutathione transferase in rats".*Toxicol*., pp. 36:71-76, 1985.

[2] Barrett, M. C., Hill, D. C., Alaxender, J. C., Zintnank, A. "Fate of orally dosed linamarin in rat". *Canadian J. of Physiol. and Phamaco.,* pp. 55:124-125, 1977.

[3] Booth, S. R., Bressani and Johns, T."Nutrient content of selected indigenous leafy vegetable consumed by Kekchi people of Alta Verapaz, Guatamela". *J. Food Compo. Anal.,* pp. 5: 25-34, 1992.

[4] Bradbury, J. H., and King, N. L. "Bitterness of cassava: Identification of new apiosylglucoside and other compounds that affect its bitter taste". *J. Sci. of food and Agric*., pp. 68:232-2230, 1995.

[5] Ellman G. and Hirolysko."A precise Method for the determination of whole blood and plasma sulfhydryl group". *Analytical Biochemistry,* pp. 93: 98-102, 1959.

[6] Esser, A., Alsen, P. and Rosling, H."Insufficient processing of cassava induced acute intoxications and the paralytic disease of Konze in rural area in Mozambique". *Ecol. Food Natr.,* pp. 27:17-27, 1959.

[7] Esser, A., Bosveld, M., Van Der Grift III, R. and Voragen A."Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen".*J. Sci. Food Agric*., pp. 63 (3): 287-296, 1993.

[8] Follet, M. J., and Ratchiff, P. W."Determination of nitrate and nitrite in meat products". *J.Sci. food Agric*., pp. 14: 138-144, 1963.

[9] Haque, M. R. and Bredbury, J. H."Simple method for determination of thiocyanate in urine".*Clin. Chem.*, pp. 45:1459-1464, 1999.

[10] Johnson, C. T. and Kross, B. C."Continuing importance of nitrate contamination of ground water and wells in rural areas". *Am. J. Ind. Ed.,* pp.18:449, 1990.

[11] Jones, T. "Poison: Nitrite/nitrates". In practice, pp.15:146-147, 1993.

[12] Liu. T. Y., Klien, C.C., and Chi, C. W. "Safrole-induced oxidative damage in liver of Sprague-Dawley rats". *Food Chem. Toxicol*., 37:697-702, 1999.

[13] Magee, P. N. "Nutrient in environmental aspect of cancer. The role of micro and macro components of food" Eds. Food nutria press, Westport, C. T., pp.198-210, 1983.

[14] Martin, F. W., and Ruberte, R."Chaya, Cnidoscolusnchayamansa includes composition and nutrition value, culture in Puerto Rico". In: Vegetable of hot humid tropics: USDA, ARS. New Orleans, LA., 1978.

[15] Meister, A. and Anderson M. E."Glutathione". *Annu. Rev. Biochem*., pp.52 (1):711-60, 1993.

[16] McVaugh, R. "The genus Cnidoscolus: generic limits and intrageneric groups". *Bul. Trrey Bot. Club*., pp. 71:457-474, 1944.

[17] Nagahara and Mendor."Utilization of sulfur-containing amino acids for cyanide detoxification". pp.13:25-78, 1999.

[18] Naqui, A., Chance, B. and Cadenas, E."Reactive oxygen intermediates in biochemistry". *Ann, Rev. Biochem.,* pp. 55:137, 1986.

[19] Philips, W.E. J. "Naturally occurring nitrite and nitrate in foods in relation to intrinsic methemoglobinemia". *Food cosmet.Toxicol*., pp. 9:219-228, 1971.

[20] Ross-Ibarra, J. and Molina-Cruze. "The ethnobotany of Chaya: A nutritioning Maya Vegetable". *Economic botany*, pp. 56(4): 350-365, 2002.

[21] Santra, A., Maiti, A., Chowdhury, A. and Mazunder, D. N."Oxidative stress in liver of mice exposed to arsenic-contaminated water". *Indian J. Gastroenter*., pp.19(3):112-115, 2000.

[22] Sorbo, B., Lundquist, P., Roshing, A. "Determination of cyanide in whole blood erythrocytes and plasma". *Clinical Chemistry,* pp. 31:591, 1953.

[23] Ulakoglu, E., GumustasBelce, A., Hug, T. and Kokoglu, E. "Alterations in superoxide dismutase activities, lipid peroxidation". *Pharmacol. Res*., pp. 38 (3):209-14, 1998.