EFFECT OF CO-ADMINISTRATION OF CASSAVA (*MANIHOT ESCULENTA CRANTZ*) RICH DIET AND ALCOHOL IN RATS

BOBY R. G. AND M. INDIRA*

*Department of Biochemistry,
University of Kerala,
Kariavattom, Thiruvananthapuram – 695 581*

Abstract : The effects of co-administration of a cassava rich diet and alcohol in rats were investigated. The animals were divided into four groups (1) Control, (2) Alcohol, (3) Cassava and (4) Alcohol + Cassava. Consumption of alcohol along with cassava reduced the alcohol induced toxicity which was evidenced by the lower activities of GOT, GPT, GGT, acid phosphatase and alkaline phosphatase in the liver and serum of co-administered group. The pyruvate content in the blood increased while the lactate content, lactate/pyruvate ratio and the activity of LDH decreased in the blood due to co-administration. The blood cyanide content, serum thiocyanate content and the activities of rhodanase and β-glucuronidase increased on co-administration. The histopathological studies also revealed that co-administration reduced the alcohol induced toxicity.

Key words :   alcohol   cassava   lactate/pyruvate ratio
                    rhodanase   acid phosphatase   GOT

INTRODUCTION

The root of cassava (*Manihot esculenta Crantz*) is a staple food for millions of people in the tropical countries. It is a good source of energy with highly digestible carbohydrates. But it has some nutritional drawbacks such as low protein content, low energy density and potential toxic effects due to the presence of cyanoglycosides-linamarin and lotaustralin. Linamarin is hydrolyzed by the bacterial β-glucosidases in the intestinal lumen resulting in the release of hydrogen cyanide. Cyanide is a metabolic inhibitor. The primary mechanism of action of cyanide involves the inhibition of cytochrome oxidase, the terminal oxidative enzymes of the electron transport chain (1). Traditional methods of cooking like boiling and decanting removes cyanoglycosides to a certain extent. But even then certain amount of residual toxicity remains in it.

Cassava is a widely grown root crop in most countries of Latin America. It ranks fourth in the list of major food crops in developing countries, after rice, wheat and maize. It is an important food item in the Toddy and Arrack shops of Kerala. Kerala

*Corresponding Author :   Phone : 0471-2418078; Fax : 0471-2307158; E-mail : indiramadambath@yahoo.co.uk

tops in the sale of liquors in India. Review of literature suggests that consumption of cassava rich diet causes several toxic effects due to the liberation of cyanide. Toxic effects of chronic alcohol consumption have been well documented (2–7). Eventhough a number of studies have been carried out on the toxic effects of cassava and alcohol, systematic studies on the toxic effects of co-administration of cassava rich diet and alcohol have been scarce. Hence it was decided to study both biochemical and histopathological effects of co-administration of cassava rich diet and alcohol in rats. Rat model was selected since its physiological system is similar to that of man and it is easier to maintain rats on synthetic diets and under different experimental conditions.

METHODS

Male albino rats (Sprague – Dawley) weighing between 100–200 g were divided into four groups of 6 rats each. Animals were housed in polypropylene cages. Cages were kept in a room that was maintained between 28°C and 32°C. The light cycle was 12 h light and 12 h dark. Animals were handled using the laboratory animal welfare guidelines (8).

Cassava (M4 variety) was purchased from the local market. It was cooked for 30 minutes and the water was decanted. This was used for the preparation of diets. The animals were fed as follows. Composition of the diet is given in Table I.

1. Control : Cassava free diet

2. Alcohol : Cassava free diet + Ethanol (4 g/kg body wt/day)

3. Cassava : Cassava diet

4. Alcohol + Cassava : Cassava diet + Ethanol (4 g/kg body wt/day)

TABLE I : Composition of the diet.

Component	Cassava-free diet (g/100 g diet)	Cassava diet (g/100 g diet)
Casein[1]	16	16
Salt mixture[2]	4	4
Ground nut oil[3]	8	8
Vitamin mixture[4]	1	1
Boiled cassava	–	71
Corn starch[5]	71	–

1. Casein for Nutritional Purpose; Spectrochem PVT LTD, Bombay.
2. Salt mixture HMW, SISCO Research Lab PVT LTD, Mumbai.
3. Postman (pure refined ground nut oil) : purchased from local market.
4. Vitamin mixture : composition is given in mg/100 g diet (Thiamin 0.8, Riboflavin 0.8, Pyridoxine hydrochloride 0.6, Niacin 5.0, Calcium pantothenate 4.0, Inositol 20.0, Folic acid 0.4, Vitamin B_{12} 2.0 µg, Retinyl acetate 1000.00 IU, Ergocalceferol 150 IU, α-tocopherol 12.0, Menadione 0.3 and Choline chloride 200.00).
5. Corn starch; SD Fine-Chem Ltd., Mumbai.

Rats were fed for 60 days. Diet and tap water were available on an *ad libitum* basis. The diet intake was recorded daily and it was found that it was almost same in all the groups. Ethanol (Ethanol 99.9–100%, "absolute" Analar and duty free, purchased from MERCK Limited, Mumbai) was given orally by gastric intubation after diluting in the ratio 1 : 1 with distilled water. The control and cassava group were given glucose solution isocaloric to that in the ethanol group. At the end of the 60 days, animals were fasted overnight and they were sacrificed. Tissues were collected in ice-cold containers for various biochemical

estimations. Blood was collected in tubes containing EDTA as anticoagulant. Serum was separated from the blood, which was collected in a tube without any anticoagulants.

Biochemical analysis

The cyanide content of boiled cassava (9), blood cyanide content (10), serum thiocyanate (11), blood lactate (12) and blood pyruvate (12) were estimated. The activities of rhodanase (EC. 2.8.1.1.) (13), β-glucuronidase (EC. 3.2.1.21) (14), lactate dehydrogenase (EC. 1.1.1.27) (15), GOT or aspartate amino transferase (EC. 2.6.1.1) (15) and GPT or alanine amino transferase (EC. 2.6.1.2.) (15), γ-glutamyl transpeptidase (GGT) (16), acid phosphatase (EC. 3.1.3.2.) (15) and alkaline phosphatase (EC. 3.1.3.1) (15) were assayed. Protein (17) in the enzyme extract was determined after TCA precipitation. The histopathological studies were carried out according to the method of Gurr E (18). Pieces of liver were fixed in buffered formalin (100 ml/L), routinely processed and embedded in paraffin wax. Sections were cut at 5 µm thickness, stained with haematoxylin and eosin and examined by light microscopy. Cell death was counted in each case.

Statistical analysis

The results were analysed using a statistical programme SPSS/PC +, Version 5.0 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA was employed for comparison among the six groups. Duncan's Post-hoc multiple comparison test of significant differences among groups were determined. $P \leq 0.05$ was considered to be significant.

RESULTS

The cyanide content of the cooked cassava was estimated to be 7–9 µg/g and that of fresh cassava was 28–32 µg/g.

The concentration of blood lactate (Table II) increased in all the treated groups in comparison with the control group ($P < 0.01$). But the lactate content was decreased in the co-administered group in comparison with alcohol and increased in comparison with cassava. The pyruvate content in the blood (Table II) was increased both in the co-administered group and in

TABLE II : Concentration of lactate and pyruvate in the blood and lactate/pyruvate ratio.

Groups	Lactate in blood (mg per 100 ml blood)	Pyruvate in blood (mg per 100 ml blood)	Lactate/Pyruvate ratio
Control	8.45±0.20	0.50±0.01	16.73±0.44
Alcohol	28.82±0.63[a]	0.32±0.01[a]	88.40±1.92[a]
Cassava	12.43±0.14[a]	0.69±0.02[a]	15.00±0.42[a]
Alcohol + Cassava	18.47±0.45[abc]	0.98±0.06[abc]	19.71±0.28[abc]

Each value is the mean (± SD) of six animals.
[a]-P<0.05 between control and treated groups.
[b]-P<0.05 between alcohol and alcohol + cassava group.
[c]-P<0.05 between cassava and alcohol + cassava group.

the cassava group in comparison with the control group (P<0.01). But there was a reduction in the alcohol group in comparison with the control group. Maximum increase was observed in the co-administered group. The lactate/pyruvate ratio (Table II) increased in the alcohol and co-administered groups when compared to the control group (P<0.01). But there was a decrease in the case of cassava group. The lactate/pyruvate ratio was decreased in the alcohol+cassava group in comparison with alcohol and increased in comparison with the cassava group.

The activities of liver and serum lactate dehydrogenases (Table III) increased significantly in all the treated groups when

compared to the group (P<0.01). The activities were significantly decreased in the co-administered group when compared to the alcohol group and significantly increased in comparison with the cassava group. The activity of β-glucuronidase (Table III) decreased significantly in all the treated groups when compared to the control group (P<0.01). But the activity increased in the co-administered group when compared to the alcohol group and decreased significantly when compared to the cassava group.

The activity of rhodanase, blood cyanide content and serum thiocyanate (Table IV) increased significantly in the cassava and

TABLE III : Activities of lactate dehydrogenase and β-glucuronidase.

Groups	Lactate dehydrogenase (μM of NAD oxidised/min/mg protein)		β-glucuronidase in the intestine (mg product formed/hr/g protein)
	Liver	Serum	
Control	2.10±0.06	20.96±0.56	77.35±2.79
Alcohol	6.96±0.13[a]	34.63±0.63[a]	40.11±1.34[a]
Cassava	2.62±0.06[a]	26.20±0.63[a]	68.82±2.28[a]
Alcohol + Cassava	3.38±0.09[abc]	29.77±0.57[abc]	57.89±1.41[abc]

Each value is the mean (± SD) of six animals.
[a]-P<0.05 between control and treated groups.
[b]-P<0.05 between alcohol and alcohol + cassava group.
[c]-P<0.05 between cassava and alcohol + cassava group.

TABLE IV : Activity of rhodanase, blood cyanide content and serum thiocyanate.

Groups	Rhodanase in liver (mg of thiocyanate formed per 100 g protein	Cyanide content in blood (μM per 100 ml blood)	Thiocyanate in serum (mg/100 ml serum)
Control	2.02±0.05	28.95±0.63	0.92±0.02
Alcohol	1.89±0.03[a]	30.02±0.74	0.99±0.03
Cassava	2.67±0.06[a]	44.37±0.84[a]	2.46±0.06[a]
Alcohol + Cassava	2.32±0.06[abc]	36.45±0.64[abc]	2.07±0.04[abc]

Each value is the mean (± SD) of six animals.
[a]-P<0.05 between control and treated groups.
[b]-P<0.05 between alcohol and alcohol + cassava group.
[c]-P<0.05 between cassava and alcohol + cassava group.

the co-administered group when compared to the control group (P<0.01). There was a significant decrease in the rhodanase activity in the alcohol group in comparison with the control group. But there was no significant change in the alcohol group in comparison with the control group. There was a significant increase in the co-administered group when compared to the alcohol group and a significant decrease, when compared to the cassava group.

The activities of GOT, GPT, GGT (Table V), acid phosphatase and alkaline phosphatase (Table VI) increased in the liver and serum of all the treated groups when compared to the control group (P<0.01). But the activities decreased significantly in the co-administered group in comparison with the alcohol group, and increased significantly in comparison with the cassava group.

The histopathological studies (Plate 1 and 2) showed that the alcohol-induced toxicity was reduced in the co-administered group in comparison with alcohol group, since the cell death was decreased in the

TABLE V :　Activities of acid phosphatase and alkaline phosphatase.

Groups	Acid phosphatase (μM of phenol lib/min/mg protein)		Alkaline phosphatase (μM of phenol lib/min/mg protein	
	Liver	Serum	Liver	Serum
Control	10.45±0.51	8.68±0.20	0.62±0.03	0.55±0.01
Alcohol	21.65±0.32[a]	18.01±0.51[a]	1.32±0.05[a]	2.40±0.06[a]
Cassava	15.02±0.38[a]	12.27±0.32[a]	0.77±0.03[a]	0.99±0.02[a]
Alcohol + Cassava	19.86±0.44[abc]	14.61±0.38[abc]	0.89±0.04[abc]	1.57±0.02[abc]

Each value is the mean (± SD) of six animals.
[a]-P<0.05 between control and treated groups.
[b]-P<0.05 between alcohol and alcohol + cassava group.
[c]-P<0.05 between cassava and alcohol + cassava group.

TABLE VI :　Activities of aspartate transaminase (GOT), alanine transaminase (GPT) and γ-glutamyl transferase (GGT).

Groups	Aspartate transaminase (μM of oxaloacetate lib/min/mg protein		Alanine transaminase (μM of pyruvate lib/ min/mg protein)		Serum GGT (μM of p-nitroaniline lib/min/mg protein
	Liver	Serum	Liver	Serum	
Control	145.22±4.43	107.53±2.54	137.40±3.19	35.32±0.64	16.08±0.44
Alcohol	261.88±2.86[a]	199.50±5.06[a]	278.44±5.07[a]	67.55±1.89[a]	32.30±0.41[a]
Cassava	180.06±2.53[a]	133.82±3.21[a]	176.44±3.17[a]	39.19±0.65[a]	18.99±0.51[a]
Alcohol + Cassava	217.77±5.08[abc]	167.45±3.82[abc]	214.08±5.69[abc]	44.25±0.78[abc]	21.92±0.57[abc]

Each value is the mean (± SD) of six animals.
[a]-P<0.05 between control and treated groups.
[b]-P<0.05 between alcohol and alcohol + cassava group.
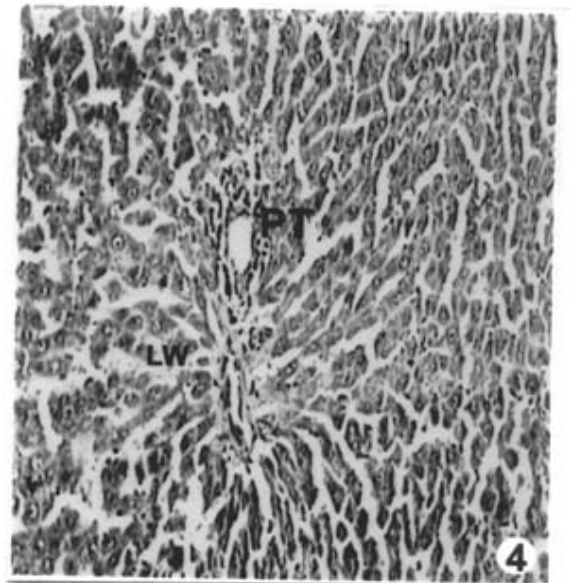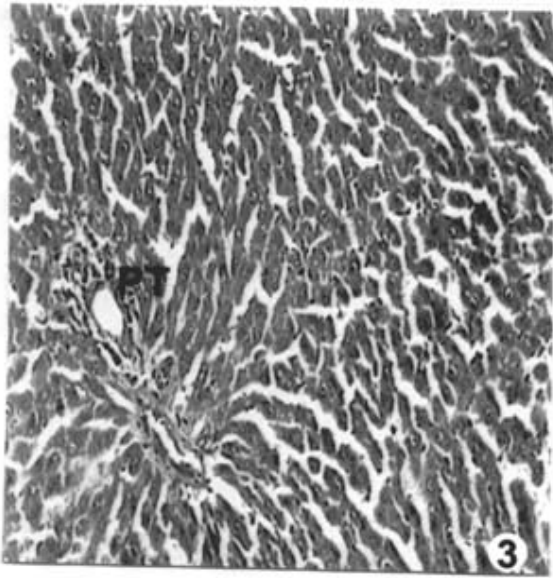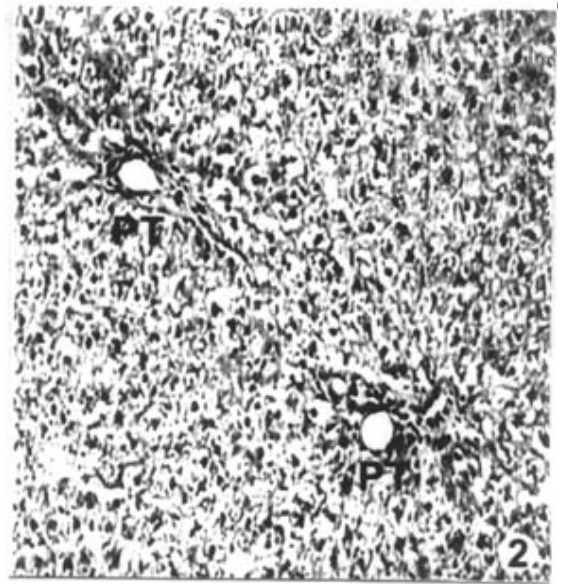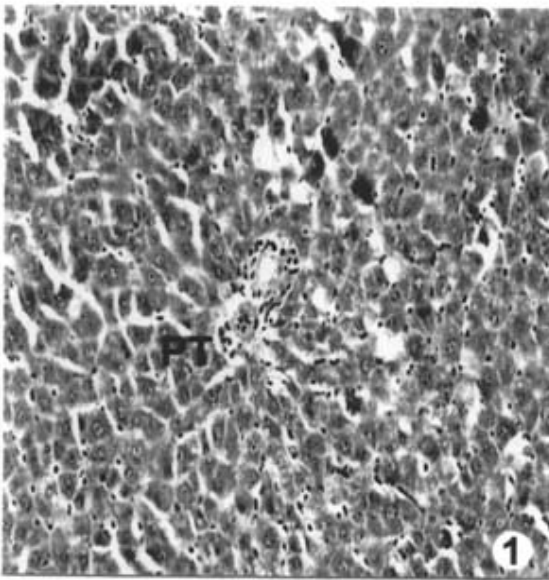[c]-P<0.05 between cassava and alcohol + cassava group.

Plate I : Light microscopic appearance of the liver sections stained with hematoxylin and eosin (HE × 200).

1. **Control** – HNE × 200
   PT–Portal Triad.
   The liver cords are normal.
   The hepatocytes show uniform
   cytoplasm and normal nuclei.

2. **Alcohol** – HNE × 200
   PT-Portal Triad.
   The picture shows shrinked two portal triads.
   The liver cords appear disrupted.
   The cytoplasm of the liver cells is vacuolated.

3. **Cassava** – HNE × 200
   PT–Portal Triad.
   Liver shows the cord like arrangement
   of hepatocytes.
   The hepatocytes are shrunken and
   the sinusoids appear markedly dilated.

4. **Alcohol + Cassava** – HNE × 200
   PT–Portal Triad.
   LW–Liver cell without nuclei.
   Liver showing marked degeneration of hepatocytes.
   The cell borders of some of the cells are undistinguished
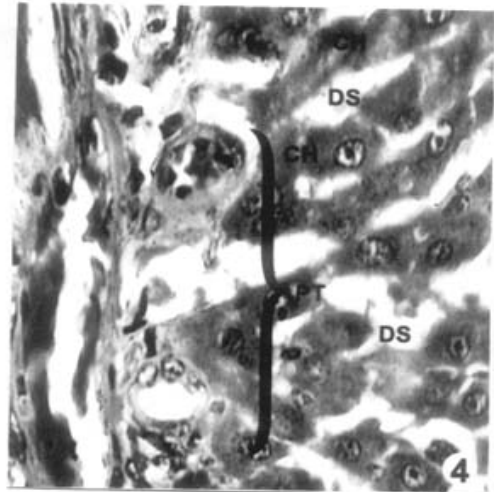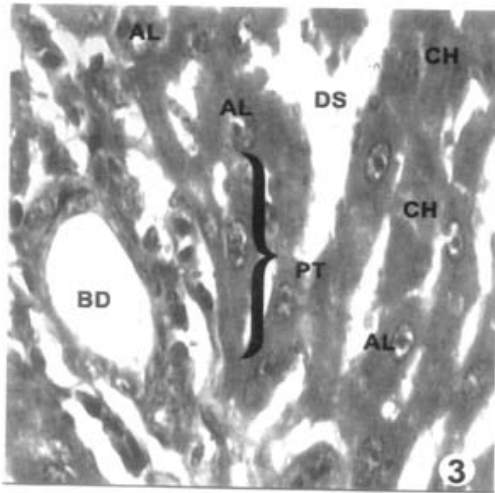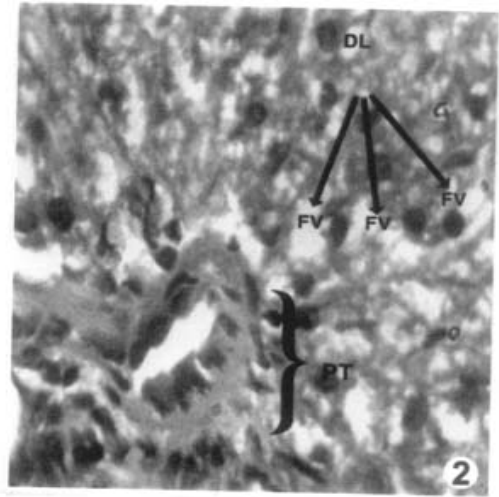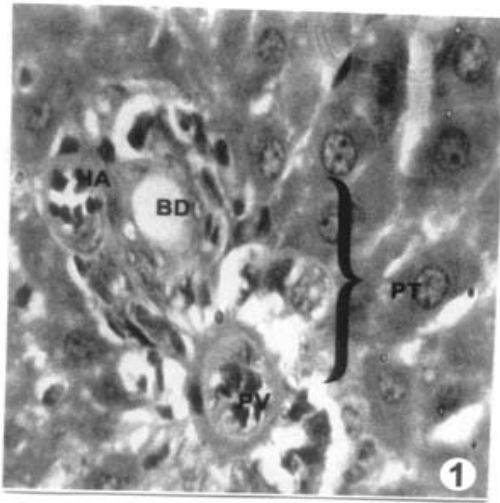   and many of them appear without nuclei.

Plate II : Light microscopic appearance of the liver sections stained with hematoxylin and eosin (HE × 400).

1. **Control** – HNE × 400
   HA–Hepatic artery.
   PV–Portal vein.
   BD–Bile duct.
   PT–Portal triad.
   Picture shows a portal triad with
   cords of hepatocytes.
   The cytoplasm around the hepatocyte is
   homogenous, the nucleus is large, spherical
   and shows prominent nuclear membrane.

2. **Alcohol** – HNE × 400
   PT–Portal triad.
   FV–Fatty vacuoles.
   DL–Degenerated liver cell.
   The figure shows a portal triad
   with surrounding liver tissue.
   The nucleus and the cytoplasm were condensed.
   The cords are disrupted, the cytoplasm
   of the hepatocytes showed multiple,
   discrete and confluent vacuoles.

3. **Cassava** – HNE × 400
   BD–Bile duct.
   AL–Atrophic liver cell.
   PT–Portal triad.
   DS–Dilated sinusoids.
   CH–Cords of hepatoytes.
   Shows the portal triad with
   cords of hepatocytes.
   The nuclei appear normal and the hepatocytes
   are atrophic causing dilated sinusoids.

4. **Alcohol + Cassava** – HNE × 400
   PT–Portal triad.
   DS–Dilated sinusoids.
   CH–Cords of hepatocytes.
   Shows disruption of the cords and
   degeneration of the hepatocytes.
   Some of the hepatocytes have lost their
   cytoplasmic borders and appear fragmented.

co-administered group. The degeneration of the hepatocytes was higher in the case of alcohol group, and there was the appearance of fatty vacuoles. Co-administration reduced the degeneration of hepatocytes and there was the absence of fatty vacuoles. But the toxicity of cassava was potentiated by alcohol administration, which was evidenced by the degeneration of hepatocytes and cell death.

DISCUSSION

In this study we used cooked cassava in which the water used for boiling was decanted off. This was designed so as to extrapolate the results to humans, since cassava is used in this part of the country similarly. But our studies showed that intake of cooked cassava induce toxicity. This supports previous studies using cooked cassava (19).

Consumption of cassava released cyanide ions by the hydrolysis of cyanoglycosides - linamarin and lotaustralin. This cyanide is the causative factor for cassava toxicity. Major route of cyanide detoxifcation in the tissue is by the enzyme rhodanase which converts cyanide to thiocyanate. So decrease in the cyanide content in the co-administered group accounts for the relative decrease in the toxicity in the co-administered group in comparison with cassava group. Profound increase in the rhodanase activity and thiocyanate content in the cassava-administered groups indicated the attempt of the tissues to detoxify cyanide. This is in agreement with the earlier reports of Padmaja (20) who studied chronic administration of sub lethal doses of linamarin to rabbits. Even though

rhodanase is an efficient antidote against the toxic effects of cyanides, there exists other pathways by which this may be mediated or assisted. Other compounds which can directly detoxify cyanide are pyruvate, mercaptopyruvate, α-ketoglutarate and ascorbate which can form cyanohydrin derivative (21).

The toxic effects of alcohol consumption are well documented (2–7). The observed elevated enzyme activities of GOT, GPT, GGT, acid and alkaline phosphatases also supports this. Hence it was expected that the consumption of alcohol along with a cassava rich diet could potentiate the toxicity induced by alcohol. But on the contrary, the co-administration reduced the toxicity of alcohol and potentiated the toxicity of cassava. The protection offered by cassava to the alcohol-induced toxicity may be due to the presence of micronutrients like vitamin C and vitamin B (22) in the cassava (22, 23). Vitamin C content of raw root was estimated (22) and found that 100 g of the raw roots contained 38.5 to 64.6 mg vitamin C while fried and boiled samples contained 29.1 to 47.4 mg and 21.5 to 40.6 mg respectively.

The alcohol intake upsets the NAD/NADH ratio. It also causes increased lactic acid accumulation and lactate pyruvate ratio. We also observed increased lactic acid and lactate/pyruvate ratio, which is in agreement with the previous reports (24). This is due to the increased activity of lactate dehydrogenase in the alcohol group. The consumption of cassava also increased the lactic acid accumulation due to the higher activity of lactate dehydrogenase. The pyruvate content in alcohol group was

decreased resulting in lactic acidosis, which was due to the generation of higher levels of NADH/NAD$^+$ due to the oxidation of alcohol. But in the cassava and in the co-administered groups, the blood pyruvate level was increased. The higher levels of pyruvate in the co-administered group in comparison with cassava may be due to the regeneration of pyruvate from lactate, which might have been accumulated in the blood due to alcohol consumption. The decreased level of lactic acid also supports this. Thus the detoxification of cyanide is increased in the co-administered group in comparison with cassava group (Fig. 1). The enhanced activity of β-glucuronidase also indicate the lower levels of toxic substances in the co-administered group when compared to alcohol group. Earlier studies had shown that the activity of β-glucuronidase is reduced in rats fed cassava rich diet (25).

The histopathological studies of the liver support our biochemical studies. It clearly showed that the hepatotoxicity induced by alcohol was reduced on co-administration of cassava. But the toxicity of cassava was potentiated by the consumption of alcohol as evidenced by the degeneration of
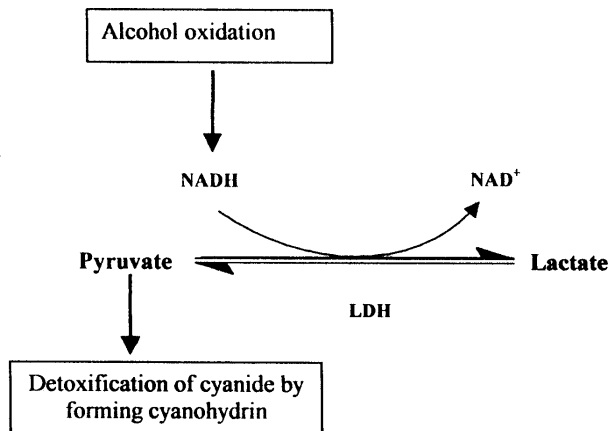

Fig. 1 : Schematic representation of the cyanide detoxification by the pyruvate formed from alcohol oxidation.

hepatocytes and cell death.

Hence it can be concluded that consumption of cassava along with alcohol reduced alcohol-induced toxicity and potentiated the toxicity of cassava as evidenced by the activities of marker enzymes and histopathological studies.

ACKNOWLEDGEMENT

REFERENCES

1. Johnson JD, William G, Conroy Kevin D, Burris Gray. Peroxidation of brain lipids following cyanide intoxication in mice. *Toxicology* 1987; 46: 21–28.

2. Niemela O, Parkkila S, Yla-Herttuala S, Villanueva J, Ruebner B, Halsted CH. Sequential acetaldehyde production, lipid peroxidation and fibrogenesis in micropig model of alcohol-induced liver disease. *Hepatology* 1995; 24: 1208–1214.

3. Schoppet M, Maisch B. Alcohol and the heart. *Herz* 2001; 26:5: 345–352.

4. Sergent O, Griffan B, Cillard P, Cillard J, Alcohol and oxidative stress. *J Pathol Biol* 2001; 49: 689–693.

5. Skrzydlewska E, Ostrowska J, Stankiewicz A, Farbiszewski R. Green tea as a potent antioxidant in alcohol intoxication. *Addict Biol* 2002; 7:3: 307–314.

6. Suresh MV, Sreeranjith Kumar CV, Lal JJ, Indira M. Impact of massive ascorbic acid supplementation on alcohol induced oxidative stress in guinea pigs. *Toxicology Letters* 1999; 104: 221–229.

7. Szuster-Ciesielska A, Daniluk J, Kandefer-Szerszen M. Oxidative stress in the blood of patients with alcohol-related liver cirrhosis. *Med Sci Monit* 2002; 8:6: CR 419–424.

8. Hume CW. The UFAW Handbook on the care and management of laboratory animals, Edinburgh/London, Churchill, Livingstone.

9. Bala Nambisan, Sukumaran Sundaresan. Spectrophotometric determination of cyanoglycosides in cassava. *J Assoc of Anal Chem* 1984; 27:3: 641–643.

10. Lundquist P, Sorbo B. Rapid determination of toxic cyanide concentration in blood. *Clinical Chemistry* 1989; 35: 617–620.

11. Lundquist P, Johannes Martensson, B. Sorbo, Sten Ohman. Method for determining thiocyanate in serum and urine. *Clinical Chemistry* 1979; 25:5: 678–681.

12. Alan H, Gowenlock, Janet R Mc Murray, Donald M Mc Lauchlan. In: Varley's Practical Clinical Biochemistry, Sixth edition, Heinemann Medical Books, London, 1988; 592–593.

13. Sorbo B, 'Rhodanase', In: Methods in enzymology, edited by SP Colowick and NO Kaplan, Academic Press, New York 1955; 2: 334–337.

14. Kawai Y, Anno K, Mucopolysaccharide degrading enzymes from the liver of the squid *Quammastrephes solani* Panclificus. *Biochem Biophys Acta* 1971; 242: 428–432.

15. Wooten IDP. Microanalysis in medical biochemistry, 4th ed., 1964, J & A Churchill Ltd. 104 Gloucester Place, London, pp. 101–115.

16. Szasz G. A kinetic photometric method for serum γ-glutamyltranspeptidase. *Clin Chem* 1969; 15: 24.

17. Lowry OH, Rosenbrough NJ, Farr AL, Randall R, Protein measurement in with Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.

18. Gurr E. Staining of animal tissues practical and theoretical, London Leonard Hill Ltd. P. 1962; 631–634.

19. Sreeja VG, Leelamma S. Effect of protein supplemented cassava diet in rats. *Indian J Biochem Biophys* 1995; 33: 149–151.

20. Padmaja G, Panikkar KR. Pattern of enzyme changes in rabbits administered linamarin or potassium cyanide. *Indian J Exp Biol* 1989; 27:6: 551–555.

21. Cittadeni A, Capprino L, Terranona T. Effect of pyruvate on the acute cyanide poisoning in mice. *Experienta* 1972; 28: 943–945.

22. Rojas I, Vitamin C en yucas peruanas Y su variacion por coccion, Lima, Peru, *Univ. Nac. Sn. marcos, yuca manihot, Turrialba,* 1967: 17: 314–316.

23. Muller Z, Chou KC, Nah KC, Tan TK. Study of nutritive value of tapioca in economic rations for growing finishing pigs in the tropics in United Nations Developmental Program (UNDP). *SF. Project SIN 67/505,* 1972; 1: 672.

24. Mascord D, Smith J, Starmer GA, Whitefield JB. Changes in lactate/pyruvate ratio in increased alcohol metabolism. *Alcohol Alcohol* 1991; 26: 53–59.

25. Sreeja VG, Leelamma S. Cassava diet – A cause for mucopolysaccharidosis ? *Plant Foods for Human Nutrition* 2002; 57: 141–150.