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

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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
trends 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>
<thead>
<tr>
<th>Component</th>
<th>Cassava-free diet</th>
<th>Cassava diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein¹</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Salt mixture²</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ground nut oil³</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Vitamin mixture⁴</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Boiled cassava</td>
<td>–</td>
<td>71</td>
</tr>
<tr>
<td>Corn starch⁵</td>
<td>71</td>
<td>–</td>
</tr>
</tbody>
</table>

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 B₃, 2.0 µg, Retinyl acetate 1000.00 IU, Ergocalciferol 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≤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.**

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

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 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lactate dehydrogenase (µM of NAD oxidised/min/mg protein)</th>
<th>β-glucuronidase in the intestine (mg product formed/hr/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>2.10±0.06</td>
<td>20.96±0.56</td>
</tr>
<tr>
<td>Alcohol</td>
<td>6.96±0.13 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.63±0.63</td>
</tr>
<tr>
<td>Cassava</td>
<td>2.62±0.06 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.20±0.63</td>
</tr>
<tr>
<td>Alcohol + Cassava</td>
<td>3.38±0.09 &lt;sup&gt;abc&lt;/sup&gt;</td>
<td>29.77±0.57</td>
</tr>
</tbody>
</table>

Each value is the mean (±SD) of six animals.

<sup>a</sup>-P<0.05 between control and treated groups.

<sup>b</sup>-P<0.05 between alcohol and alcohol + cassava group.

<sup>c</sup>-P<0.05 between cassava and alcohol + cassava group.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rhodanase in liver (mg of thiocyanate formed per 100 g protein)</th>
<th>Cyanide content in blood (µM per 100 ml blood)</th>
<th>Thiocyanate in serum (mg/100 ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.02±0.05</td>
<td>28.95±0.63</td>
<td>0.92±0.02</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1.89±0.03 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.02±0.74</td>
<td>0.99±0.03</td>
</tr>
<tr>
<td>Cassava</td>
<td>2.67±0.06 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.37±0.84 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46±0.06 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol + Cassava</td>
<td>2.32±0.06 &lt;sup&gt;abc&lt;/sup&gt;</td>
<td>36.45±0.64 &lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.07±0.04 &lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is the mean (±SD) of six animals.

<sup>a</sup>-P<0.05 between control and treated groups.

<sup>b</sup>-P<0.05 between alcohol and alcohol + cassava group.

<sup>c</sup>-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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acid phosphatase (µM of phenol lib/min/mg protein)</th>
<th>Alkaline phosphatase (µM of phenol lib/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>10.45±0.51</td>
<td>8.68±0.20</td>
</tr>
<tr>
<td>Alcohol</td>
<td>21.65±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.01±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cassava</td>
<td>15.02±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.27±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol + Cassava</td>
<td>19.86±0.44&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>14.61±0.38&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspartate transaminase (µM of oxaloacetate lib/min/mg protein)</th>
<th>Alanine transaminase (µM of pyruvate lib/min/mg protein)</th>
<th>Serum GGT (µM of p-nitroaniline lib/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Serum</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>145.22±4.43</td>
<td>107.53±2.54</td>
<td>137.40±3.19</td>
</tr>
<tr>
<td>Alcohol</td>
<td>261.88±2.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>199.50±5.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>278.44±5.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cassava</td>
<td>180.06±2.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.82±3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>176.44±3.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alcohol + Cassava</td>
<td>217.77±5.08&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>167.45±3.82&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>214.08±5.69&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is the mean (± SD) of six animals.
<sup>a</sup>-P<0.05 between control and treated groups.
<sup>b</sup>-P<0.05 between alcohol and alcohol + cassava group.
<sup>c</sup>-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 shrunken two portal triads.  
The liver cords appear disrupted.  
The cytoplasm of the liver cells is vacuolated.

3. **Cassava** - HNE × 200  
   PT–Portal Triad.  
The 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.  
The 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 hepatocytes.
   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 lotaustralrin. This cyanide is the causative factor for cassava toxicity. Major route of cyanide detoxification 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 \(\beta\)-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 \(\beta\)-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 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

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REFERENCES


