

## ETHANOL INDUCED METABOLIC ALTERATIONS AND THE EFFECT OF PHYLLANTHUS NIRURI IN THEIR REVERSAL

D.UMARANI, T. DEVAKI, P. GOVINDARAJU and K. RADHA SHANMUGASUNDARAM

*Department of Biochemistry, post-Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Madras – 600 113, India.*

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**ABSTRACT:** *Phyllanthus niruri*, Linn. (*Kizha nelli* in Tamil and *Bahupatra* and *Bhumyاملaki* in Sanskrit) which has been used widely for liver afflictions, was tried for its effectiveness in ethanol induced fatty liver, developed in rats, the increased deposition of triglyceride, cholesterol and phospholipid found in the liver, brain, kidney and heart due to ethanol administration were brought back towards the normal values on the administration of the herbal powder, however, in the intestine and stomach the increased ATPase activity observed due to alcohol ingestion, was only partially reduced.

### Introduction:

The association of liver damage with excess consumption of ethanol has been recognized in ancient Indian manuscripts dealing with Ayurvedic system of medicine<sup>1</sup>. Alcohol ingestion displays complications of liver disease to varying degrees, ranging from reversible fatty liver to alcoholic hepatitis and finally to irreversible cirrhosis<sup>2</sup>. Effects of ethanol are dose dependent affecting a wide variety of functions in the body. Acute toxicity causes progressive impairment of motor function, mental confusion and hyperexcitability while chronic toxicity causes neuropathies, heart diseases and impaired liver and kidney function and ultimately death from respiratory or cardiovascular failure<sup>3</sup>.

Ethanol affects oxidative phosphorylation in the mitochondria and there by ATP

production thus impairing the energy storing process<sup>4</sup>. ATPases, the enzymes which act on this ATP to produce energy, needed for a variety of energy dependent processes have been found to be affected variedly.

There is no satisfactory therapy yet available for the cure of this ethanol induced metabolic alterations, while still, the abstinence from alcohol and the provision of a diet with sufficient calories, vitamins and proteins are the mainstays of therapy. In this paper, we present our attempt to test the effect of the medicinal herb, *phyllanthus niruri*, Linn. which has been widely and commonly used for chronic liver diseases by the siddha physicians in the reversal of the alcohol induced metabolic alterations.

### Test drug, *Phyllanthus niruri*:

This medicinal herb, Common in central and southern India extending to Ceylon is noted

for its effectiveness in stimulating sluggish liver and is a therapy for regeneration of the liver tissue in jaundice<sup>5</sup>. This is called as Bahupatra and Bhumyaamlaki in Sanskrit, Kizha nelli in Tamil and Kizhar nelli in Malayalam. Siddha practioners give the expressed juice of the fresh plant for sluggish liver and also for chronic liver disease<sup>6</sup>. Though it is commonly and widely used, a systematic clinical study on this herb in this aspect is lacking. fore, it was decided to evaluate the effect of this herb in alcoholic fatty liver on the basis of modern scientific parameters.

### Materials and methods:

Albino rats of Wistar stock (100-130g) were maintained on a protein restricted diet (a

mixture of (5:5, w/w) rat chow and wheat flour) for a period of 30 days. This provides 10 per cent (w/w) protein and is just sufficient to keep the animals in nitrogen balance. During this period, they were given 2.5 ml of 25 per cent ethanol orally, while the controls were iso-calorically fed with a supplementation of sucrose. At the end of 30 days, the drug (200 mg/rat/day) was administered to one group of the animals for 45 days. Animals were divided into six groups as given in table 1, assess the effect of alcohol and *Phyllanthus niruri* in normal and alcohol fed conditions, the experiment was completed at the end of 75 days, which was 45 days after the initiation of drug therapy.

TABLE-I

### Experimental Set-up

Group	I Stage (30 days)	II Stage (31 <sup>st</sup> to 75 <sup>th</sup> day)		Remarks
	Dietary alcohol	Dietary alcohol	P. niruri treatment	
Ia	-	-	-	Controls
Ib	-	-	+	Toxicity, if any to normals
IIa	+	+	-	Effect of ethanol
IIb	+	+	+	Action of P. niruri, in presence of ethanol
IIc	+	-	-	Effect of ethanol withdrawal
IId	+	-	+	Additive effect if any, of P. niruri

The whole plant of *Phyllanthus niruri* including roots, were washed with water and dried in the shade without direct exposure to sun's rays. The dried material was powdered in a ball-mill and 200mg of this herbal powder was administered daily to each rat individually. The herbal powder was mixed with a small quantity of the moistened feed, made into the form of paste and fed orally. Diet was placed in the cages only after this portion was consumed.

At the end of the experimental period, the animals were sacrificed by cervical decapitation and brain, liver, kidney, heart and intestinal tissues were dissected out for further analysis. Estimations of cholesterol<sup>7</sup>, triglycerides<sup>8</sup> and phospholipid<sup>9</sup> were made in brain, liver, kidney and heart tissues. 1 per cent homogenate of stomach and intestinal tissues was he with saline were prepared in 0.1M Tris-HCl buffer of pH 7.4. In this homogenate, protein<sup>10</sup>, total ATPase<sup>11</sup>, Mg<sup>2+</sup>-dependent ATPase<sup>12</sup>, Ca<sup>2+</sup>-ATPase and HCO<sub>3</sub>-ATPase (by slight modification of Evan's procedure<sup>10</sup> were assayed. To assess the statistically significant variation caused by both the alcohol and herbal treatment, students 't' test was employed.

To assess *Phyllanthus niruri* toxicity, group Ib was compared with Ia. Group IIa was

compared with Ia to assess the effect of ethanol, and IIc was compared with IIa for the effect of ethanol withdrawal. Group IIb was compared with IIa and IIb with IIe to assess the effect of *Phyllanthus niruri* in the presence of ethanol and its withdrawal respectively.

### Results:

From Table 2, it may be seen that with ethanol ingestion cholesterol and triglyceride are found to be increase in the liver significantly ( $p < 0.001$ ) in group IIa when compared to Ia confirming fat deposition. In the animals in which alcohol was withdrawn in the second stage (group IIc,) the lipid levels are lower than in alcohol-fed group IIa. Effect of the drug can be seen by comparing IIc with IIb. The triglyceride levels are significantly reduced ( $p < 0.01$ ) when *Phyllanthus niruri* is administered (group IIc) and similar reductions are also observed in cholesterol and phospholipid contents I brain. The counteracting effect of rug on alcohol induced changes and be observed by comparing IIb with IIa. The reduction in Lipid accumulation is pronounced in the liver, suggesting that this herbal drug has lipotropic effect.

TABLE – II  
Levels of triglycerides, cholesterol and phospholipid in brain and liver of normal and experimental animals

Group	Brain lipids			Liver lipids		
	Triglyceride mg/g	Cholesterol mg/g	Phospholipid mg/g	Triglyceride mg/g	Cholesterol mg/g	Phospholipid mg/g
Ia	15.88±0.3	22.66±2.4	31.31±0.2	9.85±0.3	4.79±0.03	25.53±0.4
Ib	15.14±0.3	21.39±0.5	35.04±0.6	9.47±0.5	4.35±0.3	28.50±0.9
IIa	26.08±0.9****	33.33±1.1****	53.61±8.7****	22.37±2.9****	8.59±0.4****	23.74±0.9**
IIb	24.13±0.7***	31.31±1.6*	40.50±1.8****	11.02±0.7****	4.72±0.2****	25.44±0.8**
IIc	22.53±1.4***	26.29±0.9****	37.09±0.8****	16.12±0.3****	6.31±0.2****	25.25±0.2****
IId	18.57±1.4***	24.74±0.9*	32.12±1.7****	9.83±0.9	5.53±0.4	26.05±1.0*

The values are expressed as mean ± S.D. of five animals in each group.

Group Ia is compared with IIa, IIa with IIc, IIa with IIb and IIc with IId.

The values are significant when

\*P<0.005; \*\*p<0.02; \*\*\*P<0.01; \*\*\*\*p<0.001.

Table 3 gives the lipid levels in kidney and heart of normal and experimental animals. On comparison of Ia with IIa, it can be seen that cholesterol and triglyceride levels are doubled in both the tissues as a result of ethanol consumption. It can be seen that these levels are marginally decrease in group IIc, while *phyllanthus niruri* administration (group IId) further lowers the lipid levels and brings them closer to the normal values. Comparison of groups Ia and Ib show that *Phyllanthus niruri* administration to the normals does not produce any significant changes in the kidney and heart tissues indicating absence of toxicity.

Ethanol administration inhibits both stomach and intestinal ATPases (Table 4) as can be

that cholesterol and triglyceride levels are doubled in both the tissues as a seen from the comparison of Ia with IIa. This inhibitory effect on the intestine is more pronounced, in the case of Na<sup>+</sup>, K<sup>+</sup>-ATPase. Withdrawal of ethanol reverses this effect on the enzymes as can be seen by comparison of IIa with IIc. It can be seen that the intestinal ATPases in *Phyllanthus niruri* administration along with alcohol ingestion (group IIb) does not differ significantly from those without the herb (Group IIa). In the alcohol withdrawal group also (group IId) *Phyllanthus niruri* does not appear to have any significant effect on the intestinal and gastric (stomach) ATPase activities.

TABLE – III  
Levels of triglycerides, cholesterol and phospholipid in kidney and heart of normal and experimental animals

Group	Kidney lipids			Heart lipids		
	Triglyceride mg/g	Cholesterol mg/g	Phospholipid mg/g	Triglyceride mg/g	Cholesterol mg/g	Phospholipid mg/g
Ia	9.28±0.1	4.23±0.5	19.47±1.03	3.37±0.1	2.49±0.2	15.75±0.7
Ib	11.45±0.5	3.59±0.6	18.53±0.4	3.14±0.3	2.37±0.2	15.33±0.5
IIa	20.67±2.1****	8.85±1.1****	27.40±1.1****	6.99±0.5****	5.08±1.0****	23.71±1.3****
IIb	20.17±2.3	7.37±0.3	25.63±0.6**	6.45±0.7****	4.72±0.7****	21.44±0.9**
IIc	16.77±0.03***	7.33±0.2****	22.12±0.8****	5.68±0.07****	2.68±0.08***	18.86±0.2****
IId	14.23±1.1	5.37±0.5	22.31±0.6	4.14±0.7****	2.78±0.2	17.08±0.6****

The values are expressed as mean ± S.D. of five animals in each group.

Group Ia is compared with IIa, IIa with IIc, IIa with IIb and IIc with IId.

The values are significant when

\*P<0.0; \*\*p<0.02; \*\*\*<0.01; \*\*\*\*<0.001.

### Discussion:

Lipid accumulation after ethanol ingestion has been reported by many workers, As a consequence of hepatic ethanol oxidation, an increase in lipid synthesis and a reduction in the oxidation of fat occurs<sup>13</sup>. reduced fatty acid oxidation has been reported both in vitro and in vivo<sup>14</sup> and this is believed to be the case for the accumulation of endogenous lipids<sup>15</sup>. Lipid accumulation is also associated with an increase in hepatic NADH/NHD ratio on ethanol oxidation. It can be seen that in the liver, triglyceride or neutral fat levels are more than doubled in alcohol ingested (group IIa) animals, while cholesterol levels are increased by 80 per cent. No significant changes are seen in phospholipid levels in the liver. Deposition of large quantities of triglyceride<sup>16</sup>, and smaller increases in hepatic esterified

cholesterol and phospholipid have been observed in fatty liver following ethanol administration, although the extent to which these are increased depends on the amount and duration of ethanol consumption<sup>17</sup>.

From our studies, it can be seen that with chronic ethanol ingestion the lipid levels are enhanced not only in the liver but also in the brain, kidney and heart tissues. In brain, phospholipid and triglycerides are increased by more than 60 per cent, while increases in cholesterol is by 50 per cent. In heart and kidney both triglyceride and cholesterol levels are nearly doubled, while phospholipid show moderate increase, withdrawal of alcohol has a tremendous effect on the accumulation of lipids in tissues. However, even after 45 days, withdrawal of alcohol, triglyceride, cholesterol and phospholipid levels in the

four tissues investigated are significantly higher than the control values. The drug administered is able to bring down the elevated tissue lipid levels as can be seen from the comparisons of IIa with Ib and IIc

with IId. However, its action appears to be primarily on liver when *phyllanthus niruri* administration even in the presence of alcohol (group IIb) brings down significant lowering of triglycerides and cholesterol.

TABLE – IV  
Levels of AT Pases in Stomach and intestine of normal and experimental animals

Group	Stomach AT Pases $\mu$ moles of Pi liberated/mg protein/ 15 minutes					Intestinal AT Pases $\mu$ moles of Pi liberated/mg protein/ 15 minutes			
	Total AT Pases	Na <sup>+</sup> ,K <sup>+</sup> -AT Pases	Mg <sup>2+</sup> AT Pases	Ca <sup>2+</sup> AT Pases	HCO <sub>3</sub> <sup>-</sup> AT Pases	Total AT Pases	Na <sup>+</sup> ,K <sup>+</sup> -AT Pases	Mg <sup>2+</sup> + AT Pases	Ca <sup>2+</sup> AT Pases
Ia	18.53±1.3	5.1±0.5	14.1±0.9	15.7±1.2	27.9±1.5	47.1±2.5	13.4±1.2	36.4±1.7	66.1±2.3
Ib	16.9±0.9	4.3±0.3	12.7±1.3	17.3±4.2	22.9±1.4	46.6±1.0	12.7±0.9	37.2±2.7	64.2±1.4
IIa	14.1****±0.5	1.9***±0.3	13.03*±0.9	9.4****±1.4	10.9***±1.1	37.9****±2.3	8.7*±0.5	29.2*±1.9	48.1****±2.7
IIb	17.3*±1.7	1.3±0.3	13.3±2.7	11.96**±1.5	18.7±0.7	41.9***±2.9	9.3±0.8	32.6**±2.1	41.2*±3.04
IIc	16.13*±1.3	2.6±0.1	16.3*±0.8	10.6±1.5	15.0±2.5	38.27±3.0	6.80*±0.5	32.1±1.5	32.2±3.0
IId	17.8±1.4	1.63±0.3	16.2±1.2	12.3±2.3	19.5***±2.9	41.7**±1.6	11.16****±1.9	29.6*±3.6	42.06***±2.8

The values are expressed as mean  $\pm$  S.D. of five animals in each group.

Group Ia is compared with IIa, IIa with IIc, IIa with IIb and IIc with IId.

The values are significant when

\*P<0.5; \*\*p<0.02; \*\*\*<0.01; \*\*\*\*<0.001.

It has been reported by several<sup>18,19</sup> that ethanol alters ATPase activity which is involved in the energy linked transport of Na<sup>+</sup> and K<sup>+</sup> ions across plasma membranes<sup>(20)</sup>, apparently by competing with K<sup>+</sup> ions. Hegyvari<sup>21</sup> has observed that

the functional state of this membrane bound enzyme is dependent on the presence of specific phospholipids in the membrane. A decrease in the amount of specific phospholipids associated with the protein or an exchange with different phospholipids

will alter the enzyme properties<sup>22</sup> from the Table 4, it can be seen that the enzyme activity is lowered significantly in the stomach and intestine. *Phyllanthus niruri* administration in the normal animals do not show any marked changes in the stomach and intestinal ATPases, probably due to the fact, that it has no toxic effect on the mucosa and does not impair the normal absorptive processes in the gastrointestinal tract. though the Mg<sup>2+</sup>-dependent ATPase is affected by alcohol ingestion, the decrease is not significant which is consistent with the other reports<sup>23,24</sup>, and it is suggested<sup>25</sup> that it may be because this is not an integral part of the cell membrane. As to the decrease in Ca<sup>2+</sup>-ATPase activity, the decreased ATP synthesis after acute and chronic administration<sup>26</sup> may in turn cause a decrease in the efflux of calcium from the cells consequently the tissue calcium is increased with the accompanying reduction in the Ca<sup>2+</sup>-ATPase activity. It is also suggested that the decrease in ATP production<sup>27</sup> by ethanol may also be a reason for the lowered ATPase activity observed. Though there are reports that ethanol increases acid secretion<sup>28</sup> and HCO<sub>3</sub>-stimulated ATPases might be involved in acid secretion by aiding in the transport of HCO<sub>3</sub><sup>-</sup> ion<sup>29</sup>, we observed a significant reduction in HCO<sub>3</sub>- stimulated ATPase activity. *Phyllanthus niruri* appears to have no effect on the ATPases in the gastrointestinal tract both in the normal and also in the alcohol fed condition.

While the herb, *Phyllanthus niruri* brings down the tissue lipid levels in the alcohol fed animals, it apparently has no correcting influence on the ATPase levels in the

gastrointestinal tract. The drug is non-toxic, as it produces no significant changes in both the lipid and enzyme levels in the rats which are not given alcohol. *Phyllanthus niruri* can be considered as a safe lipotropic drug, and its primary action in on the liver.

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