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Hepatic RNA/Protein Ratio And Body Weight Of Rats Subjected To Doses Of Alcohol

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ABSTRACT

The effect of ethanol administration on rat liver sub-fractions (Whole Homogenate WH and post mitochondrial supernatant PMS), total protein content, liver RNA and RNA/protein ratio was studied. Administration of 20% ethanol as drinking water led to significant increase in rat liver weight: 19% for male rats and 43% for female rats, respectively. The mean total protein values, in the PMS were 420mg and 350mg respectively. These values were significantly higher than the control values of 420mg and 210 for male and female rats respectively, ($P<0.001$). The RNA result reflects no significant changes ($P>0.1$) compared to untreated rats, irrespective of sex. The liver (PMS) RNA for male and female test groups, were 0.010 and 0.011 respectively. These values are comparable with the control values of 0.010 and 0.012. The RNA/protein ratio obtained from ethanol pretreated rats showed a decreasing trend, which was significantly lower $P<0.01$ than the control water treated groups, irrespective of sex. The biochemical significance of these findings has been discussed.

Keywords: Ethanol, liver whole homogenate, Postmitochondrial supernatant, RNA/protein ratio, Ingestion.

It has been proposed that regular drinkers possess enhanced capacity to metabolize alcohol (Ariyoshi et al, 1970), Alcohol is largely metabolized through three pathways namely; alcohol dehydrogenase system, catalase system, and finally, the microsomal ethanol oxidizing system (MEOS) (Lieber, 1973).

Other workers, (Akintonwa, 1986 and Oesch 1973) have reported the ability of certain drugs like phenobarbitone and 3-emthyl cholanthrone to induce drug metabolizing enzymes leading to enhanced metabolism of drugs/substrates of such enzymes. For instance, phenobarbitone administration led to two fold increase in the activity of cytochrome P450 monoxygenase, this according to (Goodman et al, 1985) is the basis for the use of phenobarbitone in the alleviation of neonatal jaundice, a clinical condition precipitated by the low levels of glucoronyl transferase in affected infants.

Ethyl alcohol is a water-soluble substance, which is mainly metabolized in the liver (Winker et al, 1969). Prolonged chronic alcohol intake may lead to impaired liver function arising from fatty liver and cirrhosis, in which case, the liver loses its capacity to handle the metabolic detoxification of other foreign compounds (Lieber et al, 1970).

According to (Ariyoshi et al, 1970), ethanol administration at low doses, over a long period of time, increases the capacity of the liver to metabolize certain drugs by enhancing the activity of relevant microsomal enzymes. Recent advances in multiple drug therapy has made it important that inclusion can be predicted using the so called concomitants of enzyme induction (Akintonwa et al, 1987). These are the changes associated with the phenomenon of enzyme induction and they include proliferation of the smooth endoplasmic reticulum, increase in liver weight and increase in total protein.

The present study, is aimed at investigating the effect of chronic ethanol consumption on certain biochemical parameters like rat liver weight, total protein and RNA.

Furthermore, RNA/protein ratio, which is an index of enzyme induction will be computed and an attempt would be made to expose the possible effects of ethanol administration on microsomal drug metabolizing enzyme system.

MATERIALS AND METHODS

Chemicals: All reagents and chemicals were of analytical grade. Perchloric acid, potassium hydroxide, Bovine serum albumin, sucrose, potassium chloride, magnesium chloride were products of BDH chemicals England. Ethanol, potassium iodide, sodium potassium tartarate, chloroform, and Tris-HCL1 were from May and Baker, Dagenham, England.

Equipment: Mortar homogenizer, glass bead, coleman spectrophotometer type 20 (Sps UV/Visible), mettler weighing balance, dissecting set.

Experimental Animals: The animals used for this study were albino rats of wister strain obtained from University of Nigeria, Nsukka Animal House. Adult rats, male and female, weighing 150 180g were separated and maintained in a well-ventilated animal house, fed with Pfizer animal feed and tap water for 2 weeks acclimatization period prior to the experiments.

After acclimatization, the rats were weighing and separated in cages. They were maintained on the commercial Pfizer rat feed. Multivitamin tablets were crushed and added to the feed. Twenty percent (20%) ethanol was administered to the rats as drinking water. They were allowed to take the (20%) ethanol ad libitum throughout the duration of the experiment. The duration of exposure to alcohol was four weeks.

Preparation of Whole Homogenate

The rats were sacrificed by anaesthesia using chloroform. The liver from each rat was immediately removed and rinsed in a small clean beaker containing 25ml ice cold.

Sucrose, Tris, Kcl_2 (STKM) buffer. pH 7.9 at 4^0C. After rinsing, it was removed and blotted on a clean filter paper and weighed on another filter paper. It was then transferred into a mortar, cut into pieces with clean scissors and forceps for easy grinding. The homogenate was then washed into a 25ml volumetric flask and made up of the mark with the STKM buffer.
This formed the whole homogenate (WH).

Sub-fractionation: (CON AND STUMPE, 1976)

An aliquot of 10ml of the WH was subfractionated in a graduated test tube into 600g pellet and 600g supernatant by centrifuging at 1900rpm for 20 minutes in a centrifuge with

r = 15.5cm

$$f = \frac{S^2 r}{89{,}500}$$

Where,

f = centrifugal force

r = radial distance (cm) from centre or axis of rotation

s = speed of rotation of the motor (rpm)

The resultant 600g supernatant was centrifuged at 8000rpm for 35 minutes to obtain the post-mitochondrial supernatant (PMS)

Protein Determination

Protein was assayed by the biuret method (Donninger et al, 1972), modified by (Akintonwa et al, 1987). Aliquots of 3.9ml distilled water was added to 0.1ml of the WH and each of the subfractions. To each tube, 4.0ml biuret working reagent was added and allowed to develop at room temperature for 30 minutes Bovine serum Albumin was used as a standard. Blank was prepared by adding 4.0ml of biuret reagent. Absorbance was taken at 540nm in 1:3 dilution.

Determination of RNA

The method used was that of Fleck and Begg (1965) as modified by (Akintonwa, 1986). The principle of this method is based on the effect of potassium hydroxide on the phosphodiester bonds in RNA. Dilute acids such as perchloric acid are used to precipitate and isolate nucleic acids (DNA and RNA). When the precipitate is treated with 0.3N potassium hydroxide a 37^0C for 1hr, cleavage of all the phosphodiester bonds in RNA only occurs to yield cyclic 2^1 3^1 phosphonucleotides that hydrolyze to a mixture of nucleoside 2^1 phosphate and nucleoside 3^1 phosphate intermediates. DNA is stable under these conditions because it lacks the 2^1 3^1 phosphonucleotide intermediates, thus reacidification precipitates the alkali stable DNA, while the RNA nucleotides resulting from hydrolysis remain in solution. The solution of ribonucleotides is then measured spectrophotometrically at 260nm after centrifugation.

RESULTS

Table 1: Effect of Ethanol Administration On Rat Body Weight

	Mean Body weight. before treatment	Mean Body wt. after treatment	Percentage change in body weight
Male	150.0 ±3.5	179.5 ±2.8	19.6 19.6
Female	152.0 ±3.8	178.0 ±4.0	17.1

Values are presented as mean of 5 experiments ± Standard Deviations.

Table 1 shows the effect of ethanol administration on rat body weight. The results shows that ethanol administration leads to appreciable increase in rat body weight. This is very strange.

Table 2: Effect of Ethanol (Administration On Rat Liver Weight)

Groups	Mean liver weight	Milligrame per gram Body weight (Mg/gbw)	Percentage increase in liver weight
Male (Test)	6.50 ± 0.26	43.3	23.3
Male (Control)	5. 45 ± 0.25	31.5	
Female (Test)	7.20 ± 1.8	47.3	48.3
Female (Control)	5.05 ± 0.21	31.0	

Table 2 shows the effects of Ethanol on Rat Liver weight. The results show that Ethanol Administration leads to significant increases in rat liver weight in both male and female rats.

Table 3: Effect Of Ethanol Pretreatment On Rat Total Protein (mg) Content

Groups	Mean Total Protein Values (mg)		Percentage Differences	
	WH	PMS	WH	PMS
Male (Test)	460 ± 10	350 ± 27	43%	60%
Male (Control)	320 ± 21.5	210 ± 18		
Female (Test)	509 ± 180	420 ± 26	37%	75%
Female (Cont.)	370 ± 25	240 ± 22		

Table 3 shows the percentage liver protein content found in rats pre-treated with ethanol. The pretreated group had significantly higher liver protein values compared to the water treated control group. The values are presented as mean of 5 experiments to standard deviation.

Table 4: Effect Of Ethanol Pre-treatment On Ra Liver RNA (mg) Content

Groups	Mean RNA values (mg)		Percentage Differences	
	WH	PMS	WH	PMS
Male (Test)	0.03 ± 0.08	0.010 ± 0.002	0%	0%
Male (Control)	0.03 ± 0.66	0.01 ± 0.001		
Female (Test)	0.03 ± 0.02	0.011 ± 0.005	0%	0%
Female (Control)	0.03 ± 0.007	0.012 ± 0.008		

Table 5: Effect Ethanol Administration On Rat Liver RNA/protein

Groups	Mean RNA/protein Ratios		Percentage Decrease	
	WH	PMS	WH	PMS
Male (Test)	0.065	0.028	- 30%	-41%
Male (Control)	0.084	0.048		
Female (Test)	0.057	0.028	- 29%	- 43%
Female (Control)	0.081	0.046		

DISCUSSION

Most drug metabolizing enzymes which are located in liver microsomal fraction are inducible, for example Glucoronyl transferase, Cytochrome P450 Monoxygenase system, epoxide hydrolase, cytochrome P448, Nitroreductase, Glutathione-S- transferase etc (Oesch et al, 1973).

Some drug like phenobarbitone, have been identified as good inducers of these enzymes (Akintonwa, 1984). However, the degree of induction depends on the inducer and other factors like the genetic disposition of the subject. For instance while phenobarbitone causes 60% induction of mammalian cytochrome P450 monoxygenase enzyme, Dieldrin causes only 35% induction of the same enzyme under similar conditions (Rees, 1979).

Enzyme induction phenomenon can be investigated by direct assay of specific drug metabolizing enzymes. Besides this, some other inducers have been shown to be associated with enzyme induction, these include increase in liver weights increase in liver total protein, decrease in the RNA/protein ratio and proliferation, of smooth endoplasmic reticulum (Rees, 1979 and Akintonwa, 1986).

From the result presented in table 1, Ethanol pretreatment led to 19.6% increase in the body weight of male rats and 17.1% increase in female rats.

This result was very strange since it has been reported by (Ariyoshi et al, 1970), that acute alcohol consumption and protracted indulgence in ethanol consumption leads to loss of weight due to loss of appetite and irritation of the gastro-intestinal tract. The observed increase in body weight therefore requires further studies for full understanding of the biochemical explanations.

Table 2 depicts the effect of ethanol administration on rat liver weight. Ethanol administration either to male and female rats showed significant increases, 23.3% in male and 48.3% in female rats. These results are in consonance with the results obtained by other workers, (Utubaku, 1987 and Rees, 1984), using a model enzyme inducer-phenobarbitone. According to (Rees, 1984), most enzyme inducers cause increase in liver weight largely due to hypertrophy.

The effects of ethanol administration on rat liver total protein values is presented in Table 3. Significant percentage increase in rat liver total protein was observed in both male and female groups compared to the control groups. However, the result of the female group with a percentage increase of 75% was more pronounced compared with the male group with percentage increase of 66%.

According to (Arias et al, 1969), phenobarbitone administration caused similar increase in the total protein levels of pretreated rats. (Rees, 1979), reported that drugs like phenobarbitone are capable of increasing the rate of protein synthesis in mammals. Some proteins are synthesized and processed faster than others, for instance, phenobarbitone pretreatment causes a two fold increase in the rate of synthesis of total microsomal protein while that of cytochrome c reductase was increased three and a half times. In the case of well studied enzyme inducer like phenobarbitone, (Orrihenius et al, (1965) attributed the observed increases in total protein to the synthesis of new protein in form of enzymes.

Table 4 depicts the effect of ethanol administration on the RNA content of the whole Homogenate (WH) and the post mitochondrial supernatant. (PMS). The result does not reflect any significant changes in the RNA content irrespective of sex and subcellular fraction. The test WH and PMS values of 0.030 and 0.01 are comparable with the control values of 0.030 and 0.01 in the male rats. For the female group, the values were 0.03 and 0.011 for WH and PMS respectively. These results were equally comparable with control values of 0.030 and 0.12.

Other workers like (Orrihenius et al, 1965 and Attah, 1990) have reported a similar trend using other drugs like phenobarbitone, a model inducer and

chloramphenicol and inhibitor of nitroreductase enzyme. The relative stability of the RNA such as the one reported in the present study, could be as a result of the earlier observation by (Wright et al, 1977), that turnover is relatively stable thereby causing the RNA content to be fairly constant, despite the presence of these drugs.

The RNA/protein ratio approach has been developed for preliminary assessment of enzyme induction (Akintonwa, 1986 and Utubaku, 1987). And more recently for enzyme inhibition (Attah, 1990). The results presented in Table 5 show that the male group had RNA/protein ratios of 0.065 (WH) and 0.028 (PMS), these values were lower than the control results of 0.094 (WH) and 0.48 (PMS) representing 41% decrease. The female group equally exhibited a similar trend where the values decreased from 0.081 to 0.057 (WH) and from 0.046 to 0.028 (PMS) respectively. The percentage difference was 43%.

The biochemical basis for this concept is as follows:

1. Drug inducible enzymes are proteins which are compartmentalized in the microsomes (SER and RER) and in the cytosol (cyosolic epoxide hydrolase) and glutathione s transferase (Oesch, 1987).
2. The drug inducible enzymes (in microsomes pretreated with an inducer) causes an increase in the proteins of SER and RER while the RNA remains fairly stable (Wright et al, 1977).

Furthermore, SER proliferation which is a concomitant of enzyme induction (Orrihenius et al, 1965 and Rees, 1979), tends to decrease the RNA/protein ratio in whole Homogenate (WH) and postmitochondrial supernatant (PMS) of the liver.

From the foregoing, decreases in the RNA/protein ratio may be interpreted to be indicative of enzymes induction and increases in RNA/protein ratio of enzyme inhibition. This approach is quite simple and cheap in the assessment of enzyme induction and inhibition. However, the limitations of this method are that it does not identify the precise effect of the potential inducer or inhibitor on specific drug metabolizing enzymes. A possible correlation between the other inducers of enzyme induction viz,

1. Increase in liver weight
2. SER proliferation and
3. Increase in liver protein especially of the PMS with specific enzyme assays as well as RNA/protein profile would give a more reliable picture of the exact effect of the drugs on specific enzymes.

CONCLUSION

The fallout of the present study is at least two fold; In the absence of appropriate substrate and equipment the RNA/protein ratio in addition to other indices seem good in the assessment of common drugs. The candidate for this assessment and the present results indicate that ethanol is capable of causing significant decreases in Rat liver RNA/Protein ratio, thus it holds strong potential as a stimulant of some microsomal drug metabolizing enzymes. Therefore, careful and more judicious steps must be taken by the clinician in administering other drugs for chemotherapy. The need for proper history taking cannot overemphasized in order to avoid clinical risks of drug overdose involving patients who indulge in alcohol intake and other drugs whose metabolism could be affected by the ethanol induced enzymes.

REFERENCES

Ariyoshi T, Takabatake E and Remmer H (1970) Drug metabolism in ethanol induced fatty liver. Life Science 9:361-369

Lieber C.S (1973) The Alcoholics: Progress in research and treatment. Bourne, P.C., and Fox, R. (Ed) Academic Press, New York. Pp. 633-103.

Akintonwa D.A.A (1986) Mechanism of RNA redistribution of 17000 x g PMS after incubation at 37°C and its impact on other biochemical investigations. Biochemistry Medicine and Metabolical Biology 35: 132-138.

Oesch F (1973) Mammalian epoxide hydralases: inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from from aromatic and olefinic compounds. Xenobiotica 3: 305-340.

Goodman L.S. and Gilman A (1985) The pharmacological basis of therapeutics. 5th Ed., Macmillan publishing Company. Incorporated, New York. Pp. 102-108.

Oesch F (1987) Significance of various enzymes in the control of reactive metabolites. Archives Toxicology 60: 174-178.

Winker K, Landquist and Tygstup N (1969) The hepatic metabolism of ethanol in patients with Cirhosis of the liver. Hournal clinical laboratory invest. 23; 59-69.

Lieber C.S. and De Carli L.M (1970) Hepatic microsomal ethanol oxidizing system invitro characteristics and adaptive processes invitro. Journal Biochemistry 245:2505-2572.

Akintonwa D.A.A and Archibong E.I (1987) Significance of some unusual RNA/protein characteristics of some sub-cellular compartments of a human foetal liver. Nigerian Journal Biochemistry 4: 48-55.

Attah N.E (1990) Effect of phenobarbitone and chloramphenicol administration on rat hepatocellular whole homogenate and post mitochondrial supernatant protein profile and RNA/protein ratio Ph.D. Thesis, University of Calabar, Calabar.

Conn E.E and Stumpf P.K (1976) Outlines of Biochemistry John Wiley and Sons incorporated, New York. Pp. 600-601.

Donninger C., Huston D.H and Pickering B.A (1972) Phosphoric acid triesterglutathione alkyl transferase. Mechanism of detoxification of dimethyl phosphate triester. Biochemistry Journal 126:701-702.

Oesch F., Morris N., Daly J.W, Grelden J.E, Nebert D.W (1973) Genetic expression of the induction of epoxide hydrase and acyl hydrocarbon hydroxylase activities in the mouse by Phenobarbital Or 3 methylchrolanthene. Molecular pharmacology 9: 692-696.

Akintonwa D.A.A. (1984) Theoretical aspect of enzyme induction and inhibition leading to reversal of resistance to biocides. Journal theoretical Biology 106:79-87.

Rees D.E (1979) Review the mechanism of the microsomla drug hydroxylating system in rat liver by Phenobarbital. General pharmacology 10:314-350.

Utubaku A.B (1987) Assessment of hepatocellular drug metabolizing enzyme induction using RNA/protein ratio. M.Sc. Thesis University of Calabar, Calabar.

Arias I.M, Doyle D and Shinike R.T. (1969) Studies of the Synthesis and degradation of proteins of the endoplasmic reticulum of rat liver Journal Biological Chemistry 244:3303-3315.

Orrihenius J., Ericsson J.L.E and Ernest L. (1965) Phenobarbitone induced synthesis of the microsomal drug metabolizing system and its relationship to the proliferation of endoplasmic membrane. Journal cell biology 25:625-639.

Wright A.S. Akintonwa D.A.A. and Wooder M.F (1977) Studies on interaction of dieldrin with mammalian liver cells at sub-cellular level. Ecotoxicology and Environmental safety 1:7-16.

Wright A.S, Donninger C., Greenland R.D, Stemmer K.L and Zaron M.R. (1978). The effects of prolonged ingestion of dieldrin on the livers of male rhesus monkey. Econotoxicology and Environmental safety (4): 477 480.