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STUDIES ON ALCOHOLISM. II EFFECT OF ACUTE ETHANOL INTOXICAION ON SOME ENZYMES AND OTHER SERUM PROTEINS IN ADULT RATS

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ABSTRACT

Acute alcohol effects (4g/Kg body weight) on activities of some serum enzymes as alcohol dehy drogenase (ADH), γ-glutamyltransferase (GGT), lactate dehydrog-enase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as some protein fractions such as serum albumin, globulins and total proteins were analyzed at 2 and 6 hr post intoxication in male albino rats.

From all enzymes activities assayed only LDH and AST have shown significant increased values 2 hr after alcohol administration whereas significant increases of variable magnitudes were recorded 6 hr post intoxication. Hypoalbuminimia was observed 6 hr after acute dosage. Also, significant depletion in serum total proteins was noticed by the end of the experiment. In contrast, glubulins showed non-significant decrease at both time points of examination. The results indicated that ethyl alcohol at that massive dose level is toxic and this toxicity can be detected 6 hours post intoxication.

INTRODUCTION

Ethyl alcohol is the most commonly abused substance, creating serious physiological, medical and social problems, therefore, the

interest should be directed towards its toxicology, especially, the toxic effects of single large doses in animals and human that in some instances lead to death (Schuckit, 1979).

The rate of ethanol elimination from the body is the sum of rates of excretion in urine, breath and sweat but the great bulk of any dose of ethanol is normally removed through the metabolic oxidation in the liver and other tissues (Kalant, 1971). Consequently, som enzymatic and metabolic changes are frequently associated not only with alcohol-related organ damage but also with excessive alcohol consumption and alcoholism even without tissue injury (Salaspuro, 1987). As a matter of fact, ethanol exerts substantial effects on almost every cell (Noth and Walter, 1984) since it is a charged small polar molecules (Sato et al., 1991) that are membrane-permeable and diffuse rapidly across lipid bilayer (Anderson, 1978). Indeed, the liver is considered as the main organ involved in both metabolism and detoxificaion of alcohol, and therefore, it is one of the major targets for influence of intoxicaion with this drug (Dsouza et al., 1992), following either acute or chronic exposure (Volentin et al., 1984; Rothschild et al., 1987)

Meanwhile, synthesis and secretion of plasma proteins espeically ablumin is a characteristic function of the liver (Higgins and Borenfreund, 1986). Accordingly, an examination of the effect of acute ethanol administration on level of serum proteins in addition to some diagnostic enzymes, mainly for liver function may shed some light on toxic effects induced by this drug.

MATERIAL AND METHODS

ANIMALS.

Male albino rats, (*Rattus norvegicus*), weighing about 100-120g, purchasd from the Egyptian Organization for Biological and Vaccine Production, A.R.E., were used as experimental animals throughout the present investigation. Animals were housed in groups in wired cages and laboratory balanced diet and water were freely available.

EXPERIMENTAL DESIGN.

Fifteen animals were randomly divided into 3 groups. Animals of the first group were intraperitoneally (i.p.) injected with saline solution (0.9% NaCl), and were used as controls. Animals of the second group were i.p. injected with 20% ethanol in saline solution at a dose of 4g/kg according to Bayomy (1982). Both control and acute animals were decapitated 2 hours post-treatment. Animals of the third group were treated exactly as the animals of the second group but they were sacrificed 6 hours post intoxication.

COLLECTION OF SERUM SAMPLES

Blood samples were collected in conical glass centrifuge tubes, put for about one hour in refrigerator for clotting, sera were then separated using cooling centrifuge and stored till undertaking of biochemical analyses as described by the author elswhere (Bayomy et al., 1994).

BIOCHEMICAL ANALYSES.

Alcohol dehydrogenase (ADH), (EC 1.1.1.1), was analysed according to the method of Bonnichsen and Brink (1955) which is based on spectrophotometric measurement of the amount of NAD^+ being reduced in 3 min at pH 9.6 in the presence of excess amount of alcohole. Gamma glutamyltransferase (GGT) (EC2.3.2.2) was assayed using the method of Persijn (1976) at 30° C with L -γ- glutamyl -3-carboxy -4 nitroanilid as a substrate. Activity of lactate delydrogenase (LDH), (EC 1.1.1.27), was measured using commercial kits of human Gesellschaft fur Biochemica und Diagnostica mbH. Activities of the enzymes alkaline phosphatase (ALP), (EC 3.1.3.1), alanine aminotransferase (ALT), (EC 2.6.1.2), and aspartate aminotransferase (AST), (EC 2. 6. 1.1) and as well as concentrations of various serum proteins were measuered using an automatic multiparameter appratus, ASTRA 8 Synchron Clinical System.

STATISTICAL ANALYSIS.

Data are presented as means ± SD in tables. Student's t-test was used to evaluate statistical significance of the data according to Hine and Wetherill, (1975).

RESULTS

SERUM ENZYMES.

The results included in table I, demonstrate that acute dosage of the drug 2 hr after intoxication induced no detectable to subtle effects on activities of most serum enzymes assayed except for activities of LDH

and AST which increased significantly ($P<0.01$). Activites of all serum enzymes assayed after 6 hr of drug injection exhibited significant increases ranged between 15 to 110% over control values.

SERUM PROTEINS

Non-significant decreases in serum protein fractions were observed 2 hr after dosage. However, there were significant depressions in serum albumin and total protein contents 6 hr post intoxication amounting to 20.8 and 16.2 %, respectively ($P<0.05$) herase non-significant decrease was observed in serum globulins at that time (Table, II).

DISCUSSION

In the present study, different parameters examined indicate that most of the acute effects, of ethanol appeared at 6 hr post intoxication. Except for LDH and AST which were sensitive to ethanol injection , activities of different serum enzymes such as ADH, GGT, ALP, and ALT did not significantly alter after 2 hr of alcohol administration whereas subtle to moderate significant elevations of variable magnitudes ranging from 15 to 44% in the activities of such enzymes occurred 6 hr after ethanol intoxication. Most of the previousely mentioned enzymes are mainly located in the cytosolic component of the cells (Agrawal and Goedde, 1991) and any cellular damage would be reflected as increased serum enzyme activities

Table I : Effect of acute ethanol administration on enzymes of rat serum.

Parameter		*Time post intoxication*			
	Control	**2 hours**		**6 hours**	
	Mean ± S.D.	Mean ± S.D.	%Diff	Mean ± S.D.	%Diff
Alcohol dehydrogenase(1)	25.67 ± 3.65	28.62 ± 2.28	+11.49	31.70±3.48*	+23.49
γ-glutamyltransferase(2)	27.22 ± 2.06	26.15 ± 1.03	-3.93	31.34 ± 3.32*	+15.14
Lactate dehyrogenase(2)	577.14 ± 59.84	845.41±148.03**	+46.66	1011.75±101.11**	-75.30
Alkaline phophatase(3)	136.15 ± 1.03	147.38 ± 16.52	+8.25	195.75 ±18.55**	+43.78
Alanine aminotraferase(3)	47.50 ± 5.17	46.50 ± 7.50	-2.11	66.30 ± 6.83**	+39.58
Aspartate aminotraferase(3)	11180 ± 4.38	206.20 ± 11.00**	+84.44	234.20 ± 36.20**	+109.48

(1) Activity is expressed as U/ml; (2) U/L ; (3) IU/L n=5 animals per group; *=singnificant (P<0.05); ** = highly significant (p<0.01).

Table II : Effect of acute ethanol administration on serum proteins in rats.

		Time post intoxication			
Parameter	**Control**	**2 hours**		**6 hours**	
	Mean ± S.D.	Mean ± S.D.	%Diff	Mean ± S.D.	%Diff
Serum Albumin °	3.36±0.58	2.28 ±0.66	-16.7	2.66 ±.34 *	-20.83
Globulins °	2.57 ±0.87	2.51 ±0.55	-8.73	2.36 ±0.60	-4.36
Total Proteins °	6.15 ±0.59	5.21 ±0.76	-15.28	5.14 ±0.66 *	-16.24

° Units are expressed as g/dl; n= 5 animals per group; * Significant , $P < 0.05$.

since the release of enzymes, particularly hepatic, is commonly regarded as a toxic response due to an altered membrane permeability of the cells and therefore, these enzymes are widely used in the detection and evaluation of organ damage, especially the liver, in laboratory animals and in man (Plaa and Hewitt, 1982; Salaspuro, 1987). Meanwhile, the liver is one of the organs most severely damaged following acute and chronic ethanol exposure (Rothschild et al., 1987; Strubelt et al., 1987) since ethanol strikingly alters liver cell membranes as reviewed by Sun and Sun (1985). Moreover, plasma membrane glycoproteins assembly in the liver, following acute ethanol administration is significantly impaired (Mailliard et al., 1984).

Regarding ADH, this enzyme is found in various tissues including liver where over 90% of the ethanol ingested is oxidized to acetaldehyde (Hawkins and Kalant, 1972). The present data show that ADH was increased significantly in serum 6 hr after intoxication. Being located primarily in the cytosolic region of the cell, little damage to the cells is therefore needed to induce its release into the serum. An increase in the permeability of the liver cell membrane for this enzyme was also demonstrated after a single administration of ethanol (Nowak et al., 1962; Leevy and Baker, 1963).

With respect to GGT, it was reported that this enzyme is the most widely used laboratory marker of alcoholism and heavy drinkers and is found in many organs and tissues including liver, biliary tract, kidney, pancrease, spleen and duodenum (Nishimura and Teschke, 1983). The present study showed that this enzyme increased in serum after 6 hr of treatment with ethanol. It is usually admitted that the stimulation of hepatic GGT activity is responsible for the observed

increase in the serum (Jacquemin et al., 1990). Exposure of animals to alcohol either *in vivo* (Ideo et al, 1980) or *in vitro* (Barouki et al., 1983) induces hepatic GGT. However, the result of other authors appeared to disagree with the above findings since they found that acute alcohol consumption has no effect on serum GGT either in healthy volumteers or in patients with alcoholic liver disease (Clark et al., 1982; Gill et al., 1982 and Devgun et al., 1985). This discripancy might arise from the differences in dose applied, experimental procedures or test models.

The present results are, to a great extent, in agreement with the previousely published findings on man and animals as it was reported that voluntary administration of alcohol in the absence of any nutritional deficiency has been shown to be associated with increased serum level of LDH (Hed et al., 1972; Song and Rubin, 1972). This enzyme has shown a high sensitivity towards acute ethanol intoxication. LDH was reported to be indicative for all tissue injuries (Wilkinson, 1970) and was also elevated in serum after repeated dosage of 1 g/kg of body weight in mice (Fayed and El-Kholy, 1986). Also, dramatic increases in serum activities of creatine phosphokinase, LDH, AST and aldolase, which are indicative in this case to acute alcoholic myopathy, are frequently seen (Nygren, 1966; Nygren and Sunblad, 1971; Hed et al., 1972; Walsh and Conomy, 1977). Similarly, previous studies indicated that serum aminotransferase enzymes increase in response to alcohol intake (Bang et al., 1958). Likwise, Mendelson and Colleagues (1966)demonstrated that in nonalcoholics there was an incease in serum AST following oral administration of alcohol, but no change occurred in alcoholic subjects after similar

treatment which suggests that tissue adaptation and resistance to potential toxic effects of acute alcohol may develop in alcoholic individuals. It is assumed that alcohol itself does not result in increased activities of serum AST, therefore an increase in activity of this enzyme is almost indicative of organ damage (Salaspuro, 1987).

ALA has been considered as the most sensitive and specific test for acute hepatocellular damage (Coodley, 1971). Highly significant increase in activity of this enzyme in serum was recorded at 6 hr post intoxication. In line with the present findings are the results of Strubelt and associates (1987) who demonstrated that, in rats, *in vivo*, 1.6 g/kg ethanol injected intravenously produced significat elevations in serum ALT and Sorbitol dehydrogenase concentrations 4hr after its administration. Similarly, *in vitro* studies indicated that ethanol, at initial concentration of 2 g/l, induced and enhanced release of enzymes from isolated rat liver (Strubelt et al., 1987; Younes and Strubelt, 1987). On the other hand, it was reported that neither aminotransferases nor ALP are significantly elevated in healthy subjects with acute ethanol intake between 0.5 and 2 g/kg body weight simulating social, eveninng or weekened drinking (Bang et al., 1958;Freer and Statland, 1977) though higher acute intake (1.5- 2.0 g/kg) may cause transient increase (Bang et al., 1958). It is convenient in this context to mention that the dose used in the present study was 4 g/kg which is considered as massive acute one. Doses like this (3-4 g/kg) used by Brohult (1960) were reported to double basal AST levels in healthy volunteers and produce occasional pathological values.

One of the important functions of the liver is the synthesis and secretion of plasma proteins (Higgins and Boenfreund, 1986). With

regards to serum albumin, the results recorded showed decreases in this protein substance at both time points of the experiments. This decrease was significant 6 hr post injection of the drug. Similar results were observed concerning serum total porteins whereas globulins decreased non-significantly. These kinds of proteins especially albumin were documented to be sensitive under some experimental factors such as starvation (Bayomy, 1994a), alcohol intoxication (Farbiszewski et al., 1987 and Lakshman et al.,1989) and in response to interaction between alcohol toxicity and fasting (Bayomy, 1994b). It was documented that albumin and transferrin production decreased after acute administration of ethanol whereas albumin production was unaffected by long- term feeding of alcohol (Rothschild et al., 1971 and Jeejeebhoy et al., 1972). Such depressions were accompanied by ultrastructural damage of the liver after highly administered acute dose reaching 8 ml/kg body weight (Jeejeebhoy et al., 1972). In a study using radio- isotops, it was found that labelled leucine incorporation into secretory protiens was inhibited by 36% in ethanol- fed rats, also inhibition of synthetic rates of various secretory proteins was observed in ethanolic rats compared to their controls (Lakshman et al., 1989). It was suggested that acetaldehyde, produced due to oxidation of ethanol, may be the cause of hepatocyte injury and is suggested to be the metabolite which interferes with protein synthesis and secretion in most of the experimental models (Baraona et al, 1977; Tuma et al., 1981 and Volentin et al, 1984).

It is relevant to mention here that in parallel with the overall decrease in serum proteins recorded in the present study, an increase in hepatic total protein content was recorded under similar experimental

conditions (Abdel-Raheem et al., 1990) which may potentiate the suggestion that alcohol and/or its metabolite(s) are behind retarded protein transport from hepatocytes which ends in reduced serum protein content. Meanwhile, the present work clearly confirms the recently formulated suggestion which states that the influence of ethanol on liver cell function and viability is dose- and exposure time-dependent (Lamb et al, 1994).

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