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ORIGINAL ARTICLE

The Effects of Alcoholic Solutions of Salts of Some Metals on Some Nephrotic and Hepatic Biochemical Parameters in Male Albino Wistar Rats *In vivo*

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ABSTRACT

The presence of inorganic toxicants in beers is a major health challenge; hence, understanding their interactions with ethyl alcohol in vivo becomes imperative. This study was designed therefore to monitor the effects of simultaneous oral co-exposure of male white albino Wistar rats to alcoholic solutions (3.04% v/v ethyl alcohol) of $CdCl_2$, Pb $(CH_3OO)_2$, Fe $(NO_3)_3$ and $ZnSO_4$ continuously for seven days by analysing for some serum biochemical parameters of the liver (alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; albumin, ALB; total protein, TP) and the kidney (creatinine, Cr and urea, Ur). Fifteen rats were divided into five groups of 3 rats each and, spectrophotometric techniques were applied to assay for the parameters. Results obtained showed elevated activities/concentration of all the parameters in the tests groups ($p<0.05$) indicating damage to the organs. On the liver, the effects due to $CdCl_2$ was the worst followed by Pb $(CH_3OO)_2$, Fe $(NO_3)_3$ and $ZnSO_4$ in that order; in the case of the kidney, the trend was: $CdCl_2$, Fe $(NO_3)_3$, Pb $(CH_3OO)_2$ and $ZnSO_4$. These results suggest that salts of these metals do aggravate the toxic effects of ethyl alcohol on the organs but to different magnitudes. SPSS 15.0 statistical software was used and One Way ANOVA was chosen to analyse the data; p value = 0.05 was considered significant.

Key Words: *Urea, Creatinine, Aminotransferases, Alkaline phosphatase, Albumin, Total protein.*

INTRODUCTION

People have been consuming ethyl alcohol (ethanol) for ages owing to its availability, affordability and its being an integral part of traditions and culture among several nations including those on the African continent where native beers are routinely prepared and consumed on daily basis. Ethanol alone has adverse effects on both the liver and kidney; it causes tubular dysfunction, alters the form and structure of kidneys by making the cell membrane thick, kidney tubules become enlarged, increased urine volume which alters body's fluid level and disturbance in electrolyte balance [1]. It can acutely inhibit release of vasopressin. Ethanol drinkers are vulnerable to several kidney related problems [2]; it perturbs magnesium exchange in kidney tubules resulting in its lose in urine [1]. Ethyl alcohol causes liver cirrhosis, alcoholic hepatitis, hepatic cancers, and deficiency of vitamins B1, B2, C and folate; reduces the level of vitamin A through enhancing the activity of the liver enzymes that break it down, hence night blindness ensues; facilitates poor absorption of amino acids and proteins from the gastrointestinal tract and consequently decreased synthesis of albumin, a carrier protein in the blood. Ethyl alcohol is a known psychoactive drug affecting the normal function of the central nervous system.

At the breweries, ethyl alcohol is prepared through fermentation in metallic brewing vessels; traditionally however, the containers could be metallic, for example drums, clay pots or combination of the two. The leaching of iron from wall of containers into the beverage prepared has been reported [3]; similarly, the leaching of zinc and manganese into the beverage being brewed was also reported [4]. Exposure to cadmium (Cd) and ethanol causes depletion of iron and therefore disturbance in its metabolism. Cadmium exposure decreases the concentration of iron in serum, liver, and femur whereas ethyl alcohol decreases it in the spleen. Ethyl alcohol could increase cadmium accumulation making the organism more susceptible to iron depletion and hence consumers may be at risks of disorders in its body status.

Since established facts about leaching of some metals into alcoholic beverages prepared for human consumption are there, coupled with the fact that exposure to two or more xenobiotics occurs in the milieu or under occupational conditions, the justification for carrying out this research cannot be over emphasised. Consequently, this work was designed to monitor the interactions between ethyl alcohol and salts of some metals *in vivo*

MATERIALS AND METHODS

Materials

Equipment: Mettler balance (Mettler PM 2000 model); hot plates (Rodwell scientific instruments limited-REVOTHERM)

Preparation of solutions

Having established the mean concentration of cadmium, lead, iron and zinc in native beers to be 0.25, 1.71, 55.8, 1.97 parts per million (ppm) respectively, alcoholic solutions, containing 3.04% (v/v) ethyl alcohol, of their salts were prepared using those values.

Animal experimentation

Matured male albino Wistar rats with mean weight of 198.52g were kept in cage and fed growers mash for 72 hours to acclimatise; 24 hours prior to commencement of treatments, the animals were starved to enhance consumption of water and feed, they were grouped into five (5) groups of three (3) rats each. In all instances, animals in the test groups were orally fed 5ml alcoholic solutions of the appropriate salts of metals and growers mash obtained from Grand Cereals and Oil Mills, Jos. The control groups were fed 5ml alcoholic solution without the salts of metals; the frequency of feeding in all cases was once in every 24 hours and hence fresh feed sample was given to each group daily.

Duration of protocol

The treatment was performed over a period of seven (7) days continuously; thereafter they were sacrificed. A dessicator saturated with chloroform was used to induce anaesthesia in the animals and, with the aid of needle and syringe, blood samples were collected and transferred into clean and dry plastic sample containers by direct cardiac puncture after prior dissection of each of the animals. The samples were then spun at 8000g for 15 minutes in a refrigerated ultracentrifuge machine. The resulting sera were separated from the clotted component of blood using micropipette and were thereafter stored in the refrigerator at 10˚C until when needed for analysis. The following parameters were analysed: albumin, total protein, alkaline phosphatase, creatinine, aspartate aminotransferase, alanine aminotransferase and urea with a view to studying the effects of the treatment on each of them. Techniques applied were: [5-9 and 10] for ALT and AST; urea; creatinine; alkaline phosphatase; total proteins and albumin respectively.

RESULTS AND DISCUSSION

Table 1: Concentration of serum Urea (mMoles/litre) and Creatinine µMoles/litre) for test and control groups

	Volume of solution Administered (ml)	Control	$CdCl_2$	$Pb(CH_3COO)_2$	$Fe(NO_3)_3$	$ZnSO_4$
Urea	5 4.	790±0.77	4.881±0.81 [a]	4.804±0.24 [a]	5.433±0.63 [a]	4.790±0.63 [a]
Creatinine	5	29.372±0.44	41.129±1.10 [a]	30.930±0.82 [a]	35.395±0.61 [a]	29.920±0.78 [a]

Values are means of three determinations (SEM), n = 3

[a] Statistically significant ($p<0.05$) compared to control

Table 2: Activities of serum ALT, AST and ALP (IU) for test and control groups

	Volume of solution Administered(ml)	Control	$CdCl_2$	$Pb(CH_3COO)_2$	$Fe(NO_3)_3$	$ZnSO_4$
ALT	5	62.150±0.22	68.260±0.45 [a]	65.628±0.23 [a]	64.122±0.33 [a]	62.152±0.55 [a]
AST	5	246,228±1.01	270.244±0.65 [a]	255.412±0.4 [a] 1	246.880±1.12 [a]	246.325±0.72 [a]
ALP	5	88.284±0.93	142.365±0.91 [a]	200.202±0.76 [a]	89.003±0.97 [a]	88.310±0.22 [a]

Values are means of three determinations (SEM), n = 3

[a] statistically significant ($p<0.05$) compared to control

Table 3: Concentration of serum Total Proteins and Albumin (g/litre) for tests and control groups

	Volume of solution Administered(ml)	Control	$CdCl_2$	$Pb(CH_3COO)_2$	$Fe(NO_3)_3$	$ZnSO_4$
TP	5	65.410±0.55	71.902±0.88 [a]	65.874±0.33 [a]	65.425±0.82 [a]	65.435±0.78 [a]
ALB	5	28.338±0.62	35.776±0.74 [a]	33.996±0.46 [a]	35.419±0.92 [a]	32.902±0.19 [a]

Values are means of three determinations (SEM), n = 3

[a] Statistically significant ($p<0.05$) compared to control

From table 1 (urea): the mean difference between the tests and control groups for Cd, Pb, Fe and Zn were 0.198, 0.014, 0.643 and 4.790 ($p<0.05$) respectively whereas results for creatinine were 11.757, 1.558, 6.023 and 0.548 ($p<0.05$) in that order as well.

From table 2 (ALT), mean differences between test and control groups for Cd, Pb, Fe and Zn were 6.110, 3.481, 1.972 and 62.152 respectively; for AST the differences were 24.020, 9.184, 0.622 and 0.002 ($p<0.05$); for ALP, results were 54.084, 111.918, 0.719 and 0.026 ($p<0.05$) in that order.

From table 3 (ALB), the mean differences between test and control groups for Cd, Pb, Fe and Zn were 7.438, 5.658, 7.081 and 4.565 respectively whereas for TP, results were 6.492, 0.404, 0.015 and 0.025 respectively.

The thrust of this work was to monitor the effects of alcoholic solutions of some salts of metals on some biochemical markers of both the liver and kidney. Ethyl alcohol interferes with intracellular iron homeostasis, causing liver injury via a mechanism involving gut-derived endotoxin in wild-type mice; it leads to the development of liver injury, hepatitis, cirrhosis, malnutrition due to increased appetite as a result of its consumption, changes in the digestive system which makes absorption of nutrients difficult, interferences with body's ability to utilise vitamins, fertility abnormalities with low sperm count and impaired sperm motility in men, low plasma testosterone with low luteinising hormone and follicle stimulating hormone, high thiobarbituric acid reactive substance (TBARS). Activities of super-oxide dismutase and glutathione s-transferase are high in alcoholics but they have low glutathione, ascorbic acid, catalase, glutathione reductase, glutathione peroxidase which may be due to increased oxidative stress; ethyl alcohol induces some hepatic dysfunction by the induction of cytochrome P_{450} which may contribute significantly to alcohol-induced liver disease [11- 14].

Chemicals and trace metals such as lead (Pb), Cadmium (Cd), Iron (Fe), Zinc (Zn) are group of toxicants that are deleterious to health. Haemochromatosis is a disease condition that results as a consequence of iron overload in the body which is lethal on the liver if not taken care of at the early stage; the toxicity of zinc leads to inhibition of copper, calcium, magnesium and iron absorption, promotes folate deficiency; it also inhibits intestinal alkaline phosphatase, xanthine oxidase and lowers high density lipoprotein levels 'good cholesterol' but raises the level of low density lipoprotein 'bad cholesterol' thus enhancing the incidence of atherosclerosis [12]. Sources of potentially toxic chemicals in human foods and drinks include food utensils and packaging materials, domestic water, agrochemicals, industrial chemical wastes [15]. The major functions of kidney are to regulate the volume and composition of the fluids and electrolytes in the body, supply of nutrients to the cells, disposal of cellular wastes and homeostasis. The substances regulated by the kidneys include water, sodium ($Na^{+)}$, potassium (K^{+}), calcium (Ca^{2+}) and Phosphates (PO_4^{3-}) in the extracellular fluids surrounding the various cells. This organ is also involved in the regulation of acid base balance which is important in maintaining cell structure permeability and metabolic activity. These functions of the kidney make it vulnerable to the effects of ethyl alcohol and perhaps induce severe consequences for the functioning of the organism [17]. Results in table 1 showed increase in the concentration of urea and creatinine over the control groups. Urea is formed in the liver as waste product of proteins and amino acids metabolism that is normally excreted in the urine by the kidney. Ethyl alcohol per se has adverse effects on the kidney by inducing changes in membrane composition and lipid peroxidation; raises blood pressure which in itself is a risk factor in renal damage [13]. Despite its adverse effects on the kidney, test groups values from this study were higher ($p<0.05$) than the control, implying synergistic relationship between salts of metals and alcohol simultaneously. Ethyl alcohol increases cadmium toxicity and rats exposed to cadmium and alcohol are more vulnerable to cadmium accumulation and thus kidney damage [14]. Where there is an injury or adverse effects on the kidney, it loses its capacity to perform its biological functions and consequently, urea accumulates in the blood, acid-base balance impaired as well as normal homeostasis of electrolytes. Albeit urea is not as toxic as ammonia, it can induce the generation of reactive oxygen species which causes lipid peroxidation and hence damage cell membranes [18]. Creatinine is the breakdown product of muscle activity. Results indicated an increase in the test (alcohol solution containing salts of metals) over the control group;

elevated concentration could suggest impaired filtration system and decreased kidney functions; furthermore, continuous ingestion of ethyl alcohol has been reported to enhance the risk of renal dysfunction and end-stage renal disease [16].

The aminotransferases (transaminases) are found in tissues but more so in the liver where they catalyse transamination reactions. Injury to the liver causes them to leak out into circulation. From table 2, it can be seen that the treatments with alcoholic solutions of various salts of metals resulted in elevated activities of ALT, AST and ALP: thus indicating some form of injury to the liver. In the case of ALT and AST, $CdCl_2$ resulted in the worst effect followed by Pb $(CH_3COO)_2$, $Fe(NO3)_3$; $ZnSO_4$ had result that was similar to that of the control. In the case of ALP however, the group fed Pb $(CH_3COO)_2$ had the highest increase ($p<0.05$) in activity compared to control; this could mean impaired plasma membrane integrity, liver dysfunction, biliary obstruction, cholestasis or impaired erythrocytes biosynthesis by the bone marrow. ALP is known to be present in bones also, the site of erythrocytes biosynthesis and Pb is known to inhibit porphobilinogen synthase in the haem biosynthetic pathway; this leads to accumulation of δ-aminolevulinic acid, a metabolite toxic to the brain.

The result in table 3 (albumin and total proteins): the concentration in the tests groups were higher relative to control ($p<0.05$). Elevated total protein concentration could indicate bone marrow diseases, chronic infections and cancer. As for albumin, increased concentration could indicate heart failure and inability to utilise proteins. These increases over controls for all the parameters considered in this work point to the fact that treatment with salts of metals resulted in some degree of damage on both organs.

CONCLUSION

Base on the results obtained, there is adverse synergy between salts of metals, especially cadmium chloride and lead acetate and alcohol on both the liver and the kidney. Even though all the results for treatments were statistically significant ($p<0.05$), the severity of the treatments on the organs were not the same. Cadmium chloride had the greatest toxicity followed closely by lead acetate and then iron nitrate. Zinc sulphate had the least synergy with alcohol. By what mechanisms these metals and ethanol interact *in vivo* causing the toxic effects on the organs remains a challenge that should be studied closely. Moreover these metal salts do not exist solely on their own in the earth crust but as complexes with other salts or compounds; this opens a door for further work using alcoholic mixtures of these metal salts on experimental rats.

REFERENCES

[1]. Epstein, M. (1997). Alcohol's impact on kidney function. *Alcohol and Health Res. World*. 21(1): 84-93.
[2]. Gazuwa, S.Y., Dabak, J.D. & Ubom, G.A. (2006). Iron Contamination of Two Local Alcoholic Drinks. *J. of Med. in the Tropics*. 8 (1): 41-43.
[3]. Gazuwa, S.Y., Dabak, J.D. & Ubom, G.A. (2008). Contaminants in local alcoholic beverages: zinc and manganese contamination. *Int. J. Biol. Chem. Sci.* 2(4): 411-416.
[4]. Jurczuk, M., Brzoska, M. M., Rogalska, J & Monikszko, J. (2002). Iron body status of rats chronically exposed to Cadmium and Ethanol. *Alcohol and Alcoholism;* 38(3): 202-207.
[5]. Reitman, S & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetate transaminase and serum glutamic pyruvic transaminase. *Am. J. Clin. Pathol*. 28*:*56–63.
[6]. Natelson, S., Scott, M.L. & Beffa, C. (1951). A rapid method for the estimation of Urea in Biologic fluids. *Am J Clin Pathol;* 21: 156-159.
[7]. Bonses, R. W & Tausskay, H. H (1945). The colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* 158*:*581–591.
[8]. Kind, P. R. M & E. J. King (1954). Estimation of serum alkaline phosphatase activity by colorimetric methods. *J. Clin. Pathol.* 7*:*322.
[9]. Gornail, A.G., Bardawill, C. J. & David, M.M. (1949). Determination of serum Proteins by means of Biuret Reaction. *J Biol. Chem;* 177:751
[10]. Doumas, B.T., Watson, W & Biggs, H.G.(1971). Albumin Standards & the measurement of serum albumin with Bromocresol green. *Clin. Chim. Acta*; 31: 87-96.
[11]. Barrv, J. & Feng, P. (2002). Molcular Regulation of Iron Homeostasis and Resistance to Infection in Alcoholics. *Front. in Biosci*. 7: d1396-1409.
[12]. Jussi, K., George, A. K., Debbie, E. G., Riitta, S & Jukka, T.S. (1999). Atherosclerosis and Lipoproteins. Artherosl. *Thromb and Vas. Biol*. 9: 3001-3006.
[13]. Kono, H., Blair, U., B., Ivan, R., Hideki, F., Yoshiro, M., Ming, Y. & Ronald, G.T. (2000). Development of an intragastric enteral model in the mouse: studies of alcohol-induced liver disease using knock out technology. *J. Hepatobil. Pancreat. Surg.* 7: 395-400.
[14]. Khursheed, P.N., Enrique, B & Lieber, C.S. (1997). Polyenylphosphatidylcholine attenuate Alcohol-induced Fatty Liver and Hyperlipemia in rats. *J. Nutr.* 127(9): 1800-1806.
[15]. Okoye, Z.S.C. (2005). Food Borne Chemical Poisons: Not by Enemy alone; 20th Inaugural Lecture, University of Jos, 1-13.
[16]. Perneger, T.V., Whelton, P.K., Pudday, I.B & Klag, M. (1999). Risk of end-stage renal disease associated with alcohol consumption. *Am J Epidemiol;* 150: 1275-1281.
[17]. Subir, K.D. & Vasudevan, D.M. (2008). Alcohol Induced effects on Kidney. *Ind. J. of Clin. Biochem.,* 23(1): 4-9.
[18]. Maria, D., Xuelong, D., Haihong, Z., Allessandria, C., Luigi. M, Tiziana., T., Massimo, P., Angelo, C., Valeria, R., Jefferey., E, Michael., B & Ida, G. (2009). Urea causes the generation of reactive oxygen species. *J. Clin. Invest.*, *(*10): 1172-1177.

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