Consequences of in vivo interactions of binary mixtures of salts of metals in alcoholic milieu on the liver and kidney of male albino Westar rats

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Abstract: *Effects of alcoholic solution of binary mixtures of salts of metals on some hepatic and nephrotic parameters in animal model was monitored using spectrophotometric technique. The serum activities of ALT, AST, ALP and concentrations of ALB, TP, UR, CR were assayed. Animals were orally exposed to the solutions continually for seven, fourteen and twenty one consecutive days respectively. Results obtained showed increased activities of ALT particularly for groups fed Fe+Zn and Pb+Fe relative control (P<0.01) whereas Fe+Cd was least; for AST activity, Pb+Cd caused highest increase in activity with Fe+Zn being the least; trend for ALP activity was Pb+Cd highest while Fe+Zn caused the least effect. In the case of albumin, Fe+Cd highest whereas Fe+Zn and Pb+Cd had values lower than the control group values. In the case of urea, Pb+Cd caused the highest increase whereas Fe+Zn was the least. As for creatinine, Fe+Cd caused the highest increase whereas Fe+Zn had the least (p<0.01). Alcoholic solution of Fe+Zn generally caused marginal increase of the parameters monitored whereas other metals combinations synergistically caused duration of administration-dependent increase levels of the parameters relative control groups and therefore the integrity and functions of the organs could be compromised. InStat3 Statistical Software was used and ANOVA was chosen to analyse the data; P value 0.01 was considered significant.*
Key Words*: Alcohol, Cadmium, Iron, Kidney, Lead, Liver and Zinc.*

I. Introduction

Of all the different types, ethyl alcohol (ethanol) is the predominantly consumed alcoholic beverage. It is produced in breweries as lager beers as well as locally as native beers including *burukutu* and *pito* produced in most African communities including central Nigeria. The producers of native beers make use of metallic and clay containers as brewing media. Consequently, metals in the walls of containers could be leached into the product whose ultimate destination is the gastrointestinal tract of consumers and hence all the body where they exert their toxic effects alongside alcohol. The pH of *burukutu* and *pito* has been reported to be acidic 3.3 [1]; [2], hence reduction is very certain to take place. [3] have reported the necrotic effects of alcoholic solution of salts of cadmium, lead, and iron on the liver and kidney of experimental animals. Brewers of native beers generate money from such venture because they are relatively cheaper than lager beers. Hence, sellers of native beers enjoy consistent patronage of consumers who drink them as alternatives. It is against this background that this work was conceived.

II. Materials and Methods.

2.1 Preparation of solutions

Having established the mean levels of cadmium, iron, lead, and zinc in native beers [3], 3.04% (v/v) alcoholic solutions of lead acetate $\{Pb(CH_3OO)_2\}$, cadmium chloride $(CdCl_2)$ iron nitrate $\{Fe(NO_3)_3\}$ and zinc sulphate $(ZnSO_4)$ were prepared using the respective values. The animals in the test groups were orally fed these solutions as their drinking water but those in control groups were fed alcoholic solution without the salts of the metals. The daily volume of alcoholic solution of salts of appropriate metals given to each group was 20mls; fresh solutions were prepared for each day.

2.2 Animal experimentation

Matured male albino Wistar rats with mean weight of 198.52 were used. Twenty four (24) hours to commencement of oral administration of samples, the animals were starved to enhance consumption of water, feeds and alcoholic solutions of appropriate salts of metals. The rats were fed feed obtained from Grand Cereals and Oil Mills, Jos, once every 24 hours for each group.

2.3 Duration of protocol

The feeding experiment spanned over seven, fourteen and twenty one days respectively. A dessicator saturated with chloroform was used to induce anaesthesia in the animals after which blood samples were collected using needle and syringe by direct cardiac puncture after prior dissection of each of the animals. Blood samples were collected in clean and dry plastic sample containers. 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 new plastic droppers in each case. The sera were then stored in the refrigerator at 10˚C until when needed for analysis. The following parameters were analysed: [4] albumin (ALB), [5] total protein (TP), [6] alkaline phosphatase (ALP), [7] creatinine (CR), [8] aspartate oxaloacetate transaminase (AST), alanine aminotransferase (ALT) and urea (UR), [9]. This was aimed at studying the effects of the experiment on these hepatic and nephrotic biochemical parameters.

Table 1: Effects of alcoholic solutions of two salts of metals on biochemical markers of the liver and the kidney following 7 days of administration.

	ALT (IU)	AST (IU)	ALP (IU)	TP (g/dm^3)	ALB (g/dm^3)	UR (mMole/dm^3)	CR (μMole/dm^3)
Control	63.05±0.50	206.43±0.83	171.39±1.12	64.02±0.97	42.42±0.11	27.35±0.34	33.48±0.51
ROH+Pb+Cd	65.08±0.75[a]	240.71±0.52[a]	302.25±1.18[a]	63.27±0.77[a]	38.11±0.12[b]	37.44±1.35[a]	53.87±1.22[a]
ROH+Fe+Zn	54.31±0.73[a]	227.18±0.05[a]	170.39±0.99[a]	64.45±0.44[a]	38.37±0.23[b]	28.60±0.36[a]	33.82±1.09[a]
ROH+Fe+Cd	63.43±0.02[a]	214.43±0.02[a]	244.15±0.54[a]	78.49±0.47[a]	46.18±0.89[a]	58.64±0.11[a]	55.51±0.11[a]
ROH+Pb+Fe	67.09±0.55[a]	207.39±0.45[a]	292.72±0.06[a]	70.06±0.81[a]	42.25±0.94[b]	46.79±0.66[a]	43.17±0.33[a]

Values are means of three determinations (±SEM); n = 6
IU: International units
[a] = significantly different compared to control group values.
[b] = not significantly different compared to control group values.

Table 2: Effects of alcoholic solutions of two salts of metals on biochemical markers of the liver and the kidney following 14 days of administration.

	ALT (IU)	AST (IU)	ALP (IU)	TP (g/dm^3)	ALB (g/dm^3)	UR (mMole/dm^3)	CR (μMole/dm^3)
Control	53.67±0.21	161.52±0.16	162.72±0.92	58.68±0.11	31.94±0.07	41.72±0.16	25.27±0.41
ROH+Pb+Cd	86.08±0.09 [a]	191.22±0.15 [a]	189.25±0.03 [a]	69.12±0.04 [a]	37.11±0.01 [a]	47.59±0.01 [a]	53.33±0.01 [a]
ROH+Fe+Zn	90.10±0.03 [a]	161.65±0.05 [a]	163.37±0.11 [a]	71.13±0.81 [a]	40.12±0.06 [a]	47.28±0.04 [a]	45.12±0.11 [a]
ROH+Fe+Cd	73.16±0.04 [a]	217.22±0.07 [a]	226.26±0.08 [a]	72.25±0.03a	40.15±0.05 [a]	47.37±0.04 [a]	106.98±0.31 [a]
ROH+Pb+Fe	64.31±0.03[a]	172.22±0.07 [a]	178.38±0.04 [a]	59.89±0.07 [a]	32.15±0.03 [a]	43.54±1.31 [a]	46.57±0.12 [a]

Values are means of three determinations (±SEM); n = 6
IU: International units
[a] = significantly different compared to control group values.
[b] = not significantly different compared to control group values.

Table 3: Effects of alcoholic solutions of two salts of metals on biochemical markers of the liver and the kidney following 21 days of administration.

	ALT (IU)	AST (IU)	ALP (IU)	TP (g/dm^3)	ALB (g/dm^3)	UR (mMole/dm^3)	CR (μMole/dm^3)
Control	90.42±0.14	154.53±0.25	208.77±0.08	48.57±0.10	27.62±0.10	33.22±0.10	30.39±0.12
ROH+Pb+Cd	104.35±0.32 a	238.37±0.21 a	371.16±0.11 a	73.24±0.16 a	32.25±0.12 a	50.18±0.11 [a]	34.25±0.06 [a]
ROH+Fe+Zn	126.83±0.13 a	205.52±0.42 a	333.93±0.17 a	68.43±0.95 a	38.39±0.11 a	40.22±0.10 [a]	36.98±0.31 [a]
ROH+Fe+Cd	90.30±0.11 [a]	168.54±0.20 a	211.49±0.37 a	50.77±0.26 a	37.54±0.24 a	40.99±0.41 [a]	38.33±0.11 [a]
ROH+Pb+Fe	90.12±0.09 [a]	204.44±0.11 a	258.84±0.13 a	50.05±0.11 a	32.19±0.15 a	40.79±0.54 [a]	38.34±0.11[a]

Values are means of three determinations (±SEM); n = 6
IU: International units
[a] = significantly different compared to control group values.
[b] = not significantly different compared to control group values.

In table 1 are results following seven days of experimentation thus: Pb+Cd, Fe+Zn, Fe+Cd and Fe+Pb. Considering ALT, activity in test groups was higher than control (p<0.01) except the group fed Fe+Cd; even comparisons within test groups indicated significant increases (P<0.01). For AST, parameters in the test groups increased compared to control group values (p<0.01); also, the activity of ALP in all the test groups were higher than control (p<0.01) except for the group fed Fe+Zn where the value was lower than control group value. Considering the effects of treatment on total proteins concentration, all the test groups values were higher than

those of the control (p<0.01) except group fed Fe+Zn; in the case of albumin, effects of the test led to lower concentration in groups fed Pb+Cd, Fe+Zn, Pb+Fe compared to control group values whereas the other groups values were higher than the control group (p<0.01); for urea, all the test group values were higher than the control group value (p<0.01) so also was the case vis-a-viz creatinine concentration except where the animals were fed Fe+Zn.

Table 2 gives the results for same treatment but for fourteen (14) consecutive days; treatment resulted in elevated activities of the enzymes and concentration of all the parameters but to different magnitudes; the elevated values in test groups were statistically significant (p<0.01). Considering ALT activity, group fed Fe+Zn had the highest value whereas group fed Fe+Pb had the least increase compared to the control group (p<0.01); in the case of AST, group fed Fe+Cd accounted for the highest activity whereas group fed Fe+Zn has the least activity values (p<0.01). Considering ALP activity, all the test groups had values higher compared to control group (p<0.01) values with group fed Fe+Cd having the highest activity; group fed Fe+Pb having the least increase. In the case of total proteins, group fed Fe+Cd had the highest concentration whereas group fed Fe+Pb had the least elevation; all the increases were significant (p<0.01) compared to control. For albumin group, there were increases in test groups compared to the control (P<0.01) with alcoholic solution of Fe+Cd having the highest increase whereas group fed Fe+Pb had the least increase. The group fed Pb+Cd had the highest increased concentration in the case of urea whereas that fed Pb+Fe had the least increase albeit significant (P<0.01) relative to control group values. In the case of creatinine, all the treatments resulted in increase concentration with group fed Fe+Cd having the highest; group fed Fe+Zn had increase but not significant (p>0.01) compared to the control group values.

From table 3 where the experiment lasted 21 days, all the test groups had higher values relative the control group; group fed Fe+Zn had highest elevation whereas group fed Fe+Pb had least increase considering ALT; for AST, group fed Pb+Cd caused highest effect and group fed Fe+Pb had the least effect but all test groups elevations were significant (p<0.01). For ALP, group administered Cd+Pb accounted for the highest increase whereas group fed Fe+Cd resulted in the least increase albeit all the increases in all the test groups were statistically significant (p<0.01) relative the control group value; in the case of TP, Pb+Cd group had the highest impact whereas group fed Pb+Fe had the least impact (p<0.01) compared to the control group; in the case of ALB, group fed Fe+Zn caused the highest effect, Pb+Fe caused the least increase compared to the control group value (p<0.01); considering urea, group fed Pb+Cd accounted for the highest increase in concentration whereas those fed Fe+Zn accounted for least increase albeit result for all the test groups were statistically significant (p<0.01). The group fed Pb+Fe caused highest effect whereas group fed Pb+Cd resulted in the least effect with respect to creatinine.

III.   Discussion

Cadmium is a toxic metal causing injury and necrosis to nephrons, hepatocytes and several other cells; it preferably accumulates in the liver and kidneys [10] and was reported by [11] to be better absorbed in the presence of ethyl alcohol; it induces oxidative cellular damage in human foetal lung fibroblast with hydrogen peroxide being the reactive oxygen species involved [12], it depletes glutathione and protein-bound sulphydryl groups thus causing enhanced production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide [13].

Lead is another toxic metal implicated in free radical generation by inhibiting 5' iododinase activity in rat liver homogenate, lowering serum triiodothyronine and thyroxine (T_3 and T_4); deposited in erythrocytes, bone and teeth, kidney, liver, brain and bone marrow [14]. Its size is also similar to Zn^{2+} and Fe^{2+} ions and can therefore substitute them in some overlapping biochemical pathways thus antagonising them. It cannot be broken down to make it less toxic; [15] reported that iron supplementation of lead-exposed children did not reduce blood lead levels just as when zinc supplements were administered; therefore, neither iron nor zinc could serve as antidote for lead toxic effects. Lead inhibits the formation of porphobilinogen by inhibiting the catalytic action of δ-aminolevulinic acid dehydrase in the pathway of haem biosynthesis; it causes cellular membrane become fragile and Ca^{2+} and Fe^{2+} deficiencies increase its absorption, so do fatty diets as well as empty stomach.

Iron, is involved in several biochemical pathways; component of haem, and vital in bone marrow functions. Within the body, it exists in two oxidation states; Fe^{2+} and Fe^{3+}. It can bind to, and   form complexes with numerous macromolecules whose consequence is disruption in normal activities of the affected complexes.

Zinc plays vital role in axonal and synaptic transmission, necessary for nucleic acid metabolism and brain tubulin growth and phosphorylation [16]. However, its intake decreases copper content of various organs but high dietary iron inhibits its absorption via competition for binding with endogenous picolinic acid [17].

Considering table 3 where animals were fed alcoholic solution containing salts of cadmium and lead at 3.04% ethyl alcohol concentration (v/v) for twenty one days. Results of all the parameters indicated higher activities/concentrations for the test groups against control.

Table 2 bears results after 14 days of experimentation. It was a medley of trends. Activity of ALT at this concentration was higher (P<0.001); as for AST and ALP, activities were higher than control group values; concentration of total proteins in test group was also higher than the control. In the case of urea and creatinine, ethyl alcohol appeared to enhance their serum concentration because their levels were both higher than the control values. After 21 days of experimentation (table 3), generally all the hepatic parameters activities/concentrations were elevated; for the nephrotic parameters, urea concentration in test groups was higher than control group values which is similar for the levels of creatinine. Cadmium and alcohol are toxic to cells and their co-administration has been reported to cause urinary protein excretion which was higher than control group (p<0.001), further, following alcohol administration, there was increase in serum urea and decrease in serum total proteins accompanied by decreased urinary urea as well as activity of alkaline phosphatase[18]; they also reported that exposure to cadmium alone decreased the urinary urea level, increased urinary ALP activity as well as serum urea but had no effect on the TP concentration in serum and urine; Co-administration of Cadmium together with ethyl alcohol has been reported to cause increase in serum urea but did not affect the level of total proteins, urinary excretion of urea was markedly reduced [19]. In the case of the group fed alcoholic solution containing iron and zinc, except for ALT and ALB whose levels were similar to the control group values, the concentrations/activities of all the parameters for the test groups were higher against the control (p<0.001) following 7 days of experimentation; after 14 days of experimentation, the activities of AST and ALP were found to be similar to those of the control groups (p>0.05), further, their activities after 7 days of treatment were higher than after 14 days; following 21 days of experiment, the levels of all the parameters for all the test groups were higher compared against control group (p<0.001) and the magnitude of these increases was higher than at other duration of the analyses.

As for the group administered alcoholic solution containing iron and cadmium, the activities/concentration of all the parameters in test groups were increased above the control groups values (p<0.001), activities of ALT and AST were duration of administration dependent but severity of effects for ALP, TP, ALB and Ur were highest after 7 days of treatments; this could mean the animals' immune mechanism was induced and therefore antibodies were released over time to mitigate the effect of the treatment thus accounting for the observed decrease in the toxic effects over longer duration of time of experimentation.

With respect to animals administered alcoholic solution containing iron and lead, elevated values of the parameters in the test groups compared to control group values (p<0.001) were obtained but the changes did not appear to be duration of exposure dependent; following 7 days (table 1) of administering the solution to the rats, the activity of alkaline phosphatase in the sample was highest, the activities of the transaminases, concentration of urea, albumin and total proteins also indicated similar trend. It appears the effects of co-administration of iron and lead is not influenced by the presence or absence of alcohol. [20] have reported that alcoholic solution of lead acetate could predisposed to hypertension in rats, alcohol at low dose does not cause it although it can decrease or enhance vascular tone caused by lead; lead acetate causes increased blood flow in the bones which may contribute to the increase and maintenance of blood level. It has also been reported that lead acetate caused deleterious effects on the kidneys, decrease in cortical thickness, tubular atrophy and thickening of endothelial basement in glomeruli, degenerated nucleic in proximate and distal tubules [20].

In vivo, co-administration of cadmium and lead causes peripheral arterial disease, inhibition of the activity of superoxide dismutase, and impaired protein biosynthesis [21]; [22]. High blood lead concentration is directly proportional to alcohol consumption [23]. As for iron and zinc co-administration, since they have similar ionic configuration, they mutually compete for absorption sites also. A high level of iron in solution negatively affects zinc absorption; further, high zinc:iron ratio has been shown to inhibit iron uptake in animals [24]. In this report, co-administration of these metals consistently cause the elevation of both the serum activities and the concentrations of the enzymes and non enzymic parameters respectively in the test groups compared to control group values for both liver and kidney. The co-administration of iron and lead in this work caused adverse effects on the organs. Iron undergoes redox recycling whereas lead depletes glutathione thus forming reactive oxygen species. Iron and lead compete for absorption owing to their chemical similarities to the extent of the body systems not being able to distinguish them from each other. They also share the divalent metal transporter, DMT1, as a common transporter within the body. Alcohol is known to greatly increase the absorption of lead by damaging the body's ability to regulate the absorption of iron. In the case of iron and cadmium interactions, [25] reported that cadmium inhibits the absorption of iron in rats only at low to normal levels of dietary iron and that at high level of iron intake; the metals are largely absorbed by other noncompetitive mechanisms. [26] reported that iron enhances the toxicity of cadmium through increased ferroportin 1 expression in J774 macrophage cells via generation of reactive oxygen species. In this report, alcoholic solution of their salts caused increased levels of the parameters analysed in test groups for both the kidney and liver especially following fourteen and twenty one days of treatment. Generally, treatments resulted in increase in the activities/concentrations of the parameters analysed. The effects appear to be duration of treatment dependent although in some cases, such was not the case. Salts of the metals synergistically

aggravated the adverse effects of alcohol for all the test groups except for Fe+Pb where it did not affect the outcome of the tests performed.

IV.   Conclusion

At 3.04% ethyl alcohol concentration therefore, salts of metals do compound adverse effects of alcohol on both liver and kidneys. Toxic effects of cadmium appeared to be reduced when both zinc and iron in ethyl alcohol were administered simultaneously following seven days of experiment but beyond that duration, there was no reduction of their effects. How these metals interact with alcohol *in vivo* should apparently constitute the next line of further research work on the subject matter.

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