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Effects of Maalox Plus® Antacid and PureCal® Calcium Supplement on Physical Characteristics, Body Weight, Tissue Minerals and Histopathology of Rats Subjected to Alcohol Intoxication

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Abstract

Overconsumption of alcohol is associated with morbidity including depletion of the body's stores of magnesium and calcium. Conversely therapy with magnesium (Mg) and calcium (Ca) in the event of chronic alcoholism normalizes elevated enzyme activities. Similar information on acute alcohol intoxication is missing. This study examined the effects of Maalox plus® antacid and pureCal® calcium supplement which are rich in Mg and Ca, respectively, on the body weight, tissue magnesium and calcium together with histopathology of rats exposed to acute alcohol intoxication. Alcohol was administered orally at a dosage 5 g/kg body weight for five days and supplements for two days of the week for duration of 28 days. The animals were weighed weekly and tissues obtained at the end of the treatment regimen. Statistical comparison was done using one way ANOVA followed by Tukey's test. Alcohol ingestion leads to hypomagnesemia a condition that was reversed by both drugs. Liver histology showed that alcohol caused cellular infiltration and cytoplasmic vacuolization. For kidney there was cellular infiltration and widening of tubules. Visible improvement of the liver histology architecture was discernible in rats co-treated with Ca and Mg. These results showed that alcohol altered tissue architecture and the renal cation exchange mechanism as shown by the variation in serum Ca^2 and K levels. Maalox plus® and pureCal® alleviated the alcohol induced adverse effects. These findings allude to these drugs being useful agents with potential applications in the management of adverse effects associated with acute alcohol intoxication.

Keywords: Alcohol; Maalox plus®; PureCal®; Liver; Kidney

Introduction

Alcohol is a psychoactive drug that has proved to be socially accepted over many centuries [1]. The earliest evidence of alcohol use by man dates back to 4000 BC. In Kenya, alcohol consumption is also high and is estimated at 4.3 litres per person [2]. According to a countrywide survey about 13% of the Kenyan population does regularly consume alcohol [3]. Evidence on the deleterious effects of excessive alcohol consumption date back to 1700 BC. Alcohol consumption at a young age is very likely to lead to dependence, social harm and deterioration of health in adulthood [4].

Factors that contributed to excessive alcohol consumption are diverse. In Kenya unemployment compounded by corruption and high prevalence of 'second generation' alcoholic drinks and traditional illicit brews is fueling the alcohol menace [5,6]. When consumed in moderation alcohol minimizes the risk of contracting coronary heart disease [7]. The positive effect of alcohol on the cardiac system can be credited to alcohol elevating high density lipoprotein, fibrinolysis and endothelial function while also suppressing plasma viscosity, fibrinogen concentration, platelet aggregation and coagulation [8].

Morbidity as a consequence of excessive alcohol consumption contributes 4% to the global disease burden [9] with approximately 2.5 million deaths being caused directly by alcohol annually [10]. The toxic effect of alcohol results in damage to a variety of body organs including the liver, brain and gastrointestinal tract [11-13]. Alcohol, a xenobiotic, when consumed in excess increases susceptibility to lung and respiratory infections [14] as well as malnutrition including deficiencies of proteins, vitamins and minerals [15].

Ethanol causes electrolyte imbalances including hypomagnesemia and hypocalcaemia which in turn mediate the many toxic effects of alcohol. Within minutes of alcohol intake the body tends to excrete 260% more magnesium thus rendering alcoholics prone to magnesium deficiency [16]. Yet prior studies have opined to magnesium deficiency aggravating hepatic damage attributed to alcohol [17]. Consequences of magnesium deficiency also include hypocalcaemia, hypokalemia, cardiac and neurological manifestations [18].

The morbidity and mortality experienced by alcoholics are thus as a consequence of a shift in electrolyte balance and concurrent acid-base disorders [19]. To attain restoration of magnesium balance as well as correction of hypocalcaemia oral magnesium supplementation is necessary [20,21]. Treatment of alcoholics through administration of magnesium has been proved to normalize elevated enzyme activities such as AST, ALT and GGT and some clinically relevant parameters [22].

The oxidative properties of this xenobiotic make the liver and the kidney prone to damage since they play a vital role in its metabolism. Ethanol induced lipid peroxidation of cellular membranes plays a crucial role in hepatotoxic and nephrotoxic action of ethanol [23,24]. Alcohol metabolism also tends to cause derangement in metabolism of carbohydrates, protein and lipids, while at the same time affecting cellular signaling [25-29]. In light of the detrimental effects of alcohol pointed out above the study herein employs drug therapy with the

drugs of choice being rich in magnesium and calcium to combat toxicity.

Materials and Methods

Materials

Alcohol (Smirnoff Vodka 37.5%, UDV Kenya Ltd., Nairobi, Kenya) was purchased from the Kenya Breweries staff shop. Maalox plus® antacid (Winthrop Pharmaceuticals Ltd., Guilford, UK) suspension containing 200 mg magnesium hydroxide per 5 ml was used as the source of magnesium. Calcium supplementation was achieved through administration of pureCal® calcium (Fountil Life Sciences Ltd., Mumbai, India) that contained 160 mg calcium per 5 ml. Both the antacid and calcium supplement were procured from local chemists.

Experimental animals

A total of 50 Wistar rats aged between 7-8 weeks were used in this study. The animals were bred and housed at the Department of Biochemistry and Biotechnology animal house. They were housed in cages at conventional conditions at a temperature of 22-25°C and a 12 h light and dark cycle. The animals were fed with commercially available standard chow diet and water *ad libitum*. Animal studies were done following the ethical guidelines of the Guide for the Care and Use of Laboratory Animals (Institute of laboratory Animal Resources, 1996).

Experimental design

The animals were allocated randomly to ten groups of five animals each. The negative controls received water only while the positive control group was treated with alcohol. The other groups were first treated with alcohol followed by Maalox plus® antacid, or pureCal® calcium or a combination of both. Details of the treatment regimens are as presented in Table 1. Alcohol was administered at a dose 5 g/kg body weight for five days in a week. The drugs were administered for the subsequent two days after alcohol ingestion [30]. Maalox plus was diluted 1:40, 1:20 and 1:10 to achieve dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg magnesium, respectively. On the other hand pureCal® was diluted 1:32, 1:16 and 1:8 to come up with dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg calcium, respectively. All the solutions were administered through the oral gavage [31] using a cannula and treatment was continued for 28 consecutive days (Table 1).

Physical parameters

Behavioural characteristics, food and water intake and body condition were studied during the treatment period. Weekly body weight for each animal was also recorded.

Sampling

48 h after the final day of alcohol administration the animals were euthanized using ether. Liver and kidney tissues were harvested for histological analysis while bone and muscle tissues were collected for tissue mineral analysis.

Tissue analysis

The hind limb was disarticulated and femur cleaned of soft tissues using hydrogen peroxide [32]. Thigh muscle was also obtained from the hind limb. The tissues were then ashed overnight in a kiln at 500°C and dissolved in 10% nitric acid [33]. Calcium and magnesium levels in the solution derived from the bone and muscle tissues were determined using flame atomic absorption spectrometry [34]. Standards for calcium and magnesium were prepared from calcium nitrate and magnesium nitrate hexahydrate, respectively, and a calibration curve was generated.

Group	Treatment
A	Water (negative control)
B	5 g/kg alcohol (positive control)
C	5 g/kg alcohol+4.25 mg/kg magnesium
D	5 g/kg alcohol+8.5 mg/kg magnesium
E	5 g/kg alcohol+17 mg/kg magnesium
F	5 g/kg alcohol+4.25 mg/kg calcium
G	5 g/kg alcohol+8.5 mg/kg calcium
H	5 g/kg alcohol+17 mg/kg calcium
I	5 g/kg alcohol+4.25 mg mg/kg magnesium+4.25 mg/kg calcium
J	5 g/kg alcohol+4.25 mg mg/kg magnesium+8.5 mg/kg calcium

Table 1: Details of the treatment regimen.

Histopathological examination

Liver and kidney were excised and fixed in 10% formalin. The liver was washed in 70% alcohol while the kidney was washed in tap water. The tissues were then dehydrated in ascending grades of alcohol [35]. They were then cleared in xylene and embedded in paraffin wax. The specimens were cut into sections of 5 µm using a rotary microtome and samples transferred to a microscope slide. Specimens were stained with haematoxylin and eosin [36]. Histological analysis was performed under Bresser LCD Micro light microscope at X10. The liver sections were examined for cytoplasmic vacuolization, inflammatory vacuolization, inflammatory infiltration, steatosis, hepatocellular necrosis and nuclear disintegration [30]. The kidney sections were examined for cellular infiltration, tubular lumen widening, haemorrhage, tubular cast, tubular degeneration, glomerular shrinkage and glomerular degeneration [35].

Data management and statistical analysis

Data on the body weight and tissue minerals level was obtained and results were presented as means ± standard error of mean. To determine statistical significance difference between the ten experimental groups, One-way Analysis of Variance (ANOVA) was performed. This was subsequently followed by Tukey's post hoc test for multiple comparisons for comparison between individual groups and to determine significant difference between means differed ($p<0.05$). All statistical analysis was performed using WINKS Statistical Data Analysis (SDA) and graphs software. Data was presented as text, tables and figures.

Results

Effects of alcohol on behavioural characteristics of rats

During the first week of the study the alcohol treated rats were less active; they had difficulty in walking and went to sleep usually huddled together in one corner of the cage. The animals were also of poor appetite and had a rough coat. From the second week onwards they started exhibiting less sluggishness and as time progressed they were seen to be more active after alcohol intake and could be seen feeding. The negative control rats on the other hand were alert and active, had a smooth coat and had a good appetite throughout the study (Figure 1).

Figure 1: Behavioural characteristics of rats. A: Control rats (alert), B: Rats are subdued and huddled together after administration of alcohol.

Effects of treatments on body weight

For the whole period of the study the animals were given standard feed and water *ad libitum*. The negative controls received no other treatment; positive controls were treated with alcohol for five days of the week while the rest received alcohol for five days plus different doses of Maalox plus® antacid and pureCal® calcium for the remaining two days of the week. The mean weight of the animals is presented in Table 2 and it shows a consistent weekly gain across all the groups. The mean weight of the all the animals at the start of the experiment was 110.38 g and 185.32 g at the end representing a 68% increase in weight over a 4 week period (Table 2).

Tukey's post hoc test for multiple comparisons was carried out to determine whether there significant differences in weight over the four week treatment period. For the negative control there was significant ($p<0.05$) weight gain in week 1 but for alcohol treated group the weight change was insignificant ($p<0.05$). From week 2 to 4 both groups recorded significant ($p<0.05$) weight gain.

When the alcohol-fed rats were treated with Maalox plus®, which is rich in magnesium, or pureCal®, which is rich in calcium, there was insignificant ($p>0.05$) weight change in the first two weeks (Table 2). The group that received 8.5 mg/kg magnesium recorded significant ($p<0.05$) weight increase in week 4. When the dose was doubled significant ($p<0.05$) weight increase was recorded in both week 3 and 4. For calcium, there was no significant weight change over the 4 weeks period. When treatment was offered as a combined dose of both drugs, the body weight of the rats on the fourth week revealed significant difference (Table 2).

	NC	PC	4.25 Mg	8.5 Mg	17 Mg	4.25 Ca	8.5 Ca	17 Ca	4.25 Mg+Ca	4.25 Mg ± 8.5 Ca
Week 0	93.80 ± 11.10	106.40 ± 8.62	57.51 ± 69.14	84.00 ± 21.87	111.40 ± 20.06	114.20 ± 43.97	120.80 ± 40.62	118.40 ± 44.84	112.80 ± 30.87	110.40 ± 33.20
Week 1	124.00 ± 8.00*	129.40 ± 16.65	73.03 ± 79.72	100.20 ± 24.90	136.00 ± 26.66	131.00 ± 42.60	129.60 ± 45.07	127.40 ± 51.95	129.40 ± 32.04	129.60 ± 37.33
Week 2	150.80 ± 12.81*	145.80 ± 16.72*	81.26 ± 91.27	107.60 ± 27.37	146.40 ± 35.22	146.00 ± 35.92	143.00 ± 51.11	138.00 ± 52.98	144.20 ± 40.44	151.80 ± 39.24
Week 3	175.20 ± 16.86*	162.00 ± 19.97*	90.99 ± 100.43	132.60 ± 25.74	176.00 ± 30.85*	158.60 ± 31.41	152.80 ± 45.87	141.00 ± 48.23	170.60 ± 45.00	174.60 ± 39.36
Week 4	196.60 ± 17.60*	181.40 ± 20.38*	100.89 ± 113.86	159.00 ± 29.44*	206.00 ± 27.50*	177.00 ± 29.66	175.60 ± 51.37	179.40 ± 54.84	195.20 ± 46.69*	195.80 ± 30.10*

Table 2: Comparison of mean weight of rats over a four week period. The p values are for multiple comparisons between the initial weight and that of four different weeks. *$p<0.05$; NC: Normal Control; PC: Positive Control; 4.25 mg=4.25 mg/kg Magnesium; 8.5 mg=8.5 mg/kg Magnesium; 17 mg=17 mg/kg Magnesium; 4.25 Ca=4.25 mg/kg Calcium; 8.5 Ca=8.5 mg/kg Calcium; 17 Ca=17 mg/kg Calcium; 4.25 Mg+4.25 Ca=4.25 mg/kg Magnesium+4.25 mg/kg Calcium; 4.25 Mg+8.5 Ca=4.25 mg/kg Magnesium+8.5 mg/kg Calcium.

Calcium and magnesium levels in muscle and femur of rats

As expected magnesium and calcium levels were higher in bone than muscle. In the untreated group the calcium levels were 17 times higher while magnesium was 1.6 times higher in bone compared to the muscle tissue. Following alcohol treatment apart from the positive control, calcium was 21 times higher in bone than muscle while magnesium was 3.6 times (Table 3).

Administration of alcohol in rats was found to lower the level of minerals in tissues but this was only significant ($p<0.05$) for magnesium in bone. Treatment of the rats with Maalox plus® or pureCal® was found to have reversed the alcohol induced hypomagnesaemia in bone (Table 3).

Effects of alcohol, Maalox plus® and pureCal® on histopathology of the liver tissues

Alcohol administration resulted in only mild tissue pathology and this finding is consistent with the levels of liver enzymes. The pathology displayed by the animals exclusively treated with alcohol was the infiltration of inflammatory mononuclear and single neutrophils cells; and cytoplasmic vacuolization. Liver tissues of the experimental group that received magnesium and calcium in a 1:2 ratio were free of this pathology. Those that received magnesium at 8.5 mg/kg did not show cytoplasmic vacuolization. While those that received calcium at 4.25 mg/kg and 17 mg/kg and those that were given combined doses of magnesium and calcium in a 1:1 ratio were devoid of cellular infiltration (Figure 2 and Table 4).

	Muscles		Bones	
	Magnesium (ppm)	Calcium (ppm)	Magnesium (ppm)	Calcium (ppm)
Negative control	5.70 ± 1.35	8.88 ± 4.65	8.53 ± 0.35	148.71 ± 10.79
Positive control	4.10 ± 1.41	1.13 ± 5.56	6.36 ± 1.88*	135.29 ± 11.23
4.25 mg/kg Mg	5.46 ± 0.53	13.49 ± 3.48	7.83 ± 1.15	135.13 ± 23.22
8.5 mg/kg Mg	5.04 ± 1.17	13.46 ± 7.58	7.04 ± 0.90	111.88 ± 11.54
17 mg/kg Mg	5.85 ± 0.60	13.29 ± 3.99	7.45 ± 0.42	131.63 ± 4.90
4.25 mg/kg Ca	4.37 ± 1.76	9.29 ± 4.96	6.79 ± 1.54	119.71 ± 21.46
8.5 mg/kg Ca	6.03 ± 0.91	10.46 ± 5.30	7.83 ± 0.70	140.71 ± 6.09
17 mg/kg Ca	5.05 ± 1.32	10.79 ± 4.89	8.17 ± 0.32	134.21 ± 31.66
4.25 Mg+4.25 Ca	5.41 ± 0.76	14.21 ± 5.34	6.84 ± 0.61	116.13 ± 19.05
4.25 Mg+8.5 Ca	7.13 ± 0.33	15.29 ± 4.09	7.81 ± 0.42	135.96 ± 16.87

Table 3: Comparison of magnesium and calcium levels in tissues of Winstar rats subjected to treatment regimens of alcohol, magnesium and calcium. The statistical comparison was between the negative control and the other groups; *p<0.05; Mg: Magnesium; Ca: Calcium.

The liver tissues of the animals that were fed alcohol did not show any signs of fat infiltration in the liver cells. However, the animals treated with 4.25 and 8.5 mg/kg of calcium had mild to moderate steatosis. Other common liver pathology associated with chronic liver disease such as focal hepatocellular necrosis and nuclear disintegration were not evident in this study (Figure 2 and Table 4).

Effects of alcohol, Maalox plus® and pureCal® on histopathology of the kidney tissues

The kidney tubules of the negative control exhibited normal histological features. While the alcohol treated group showed mild tissue pathology and this finding is consistent with the findings on BUN and creatinine levels. The pathology displayed by the animals treated with alcohol only was cellular infiltration and widening of the tubular lumen (Figure 3 and Table 5).

Treatment with Maalox plus® and pureCal® was found to have no influence on cellular infiltration or the widening of the lumen of the kidney tubules. Magnesium treatment was associated with haemorrhage and so was calcium at 8.5 mg/kg and 17 mg/kg. Calcium at 17 mg/kg was also associated with tubular cast formation and tubular degeneration. No pathology was associated with the glomerulus (Figure 3 and Table 5).

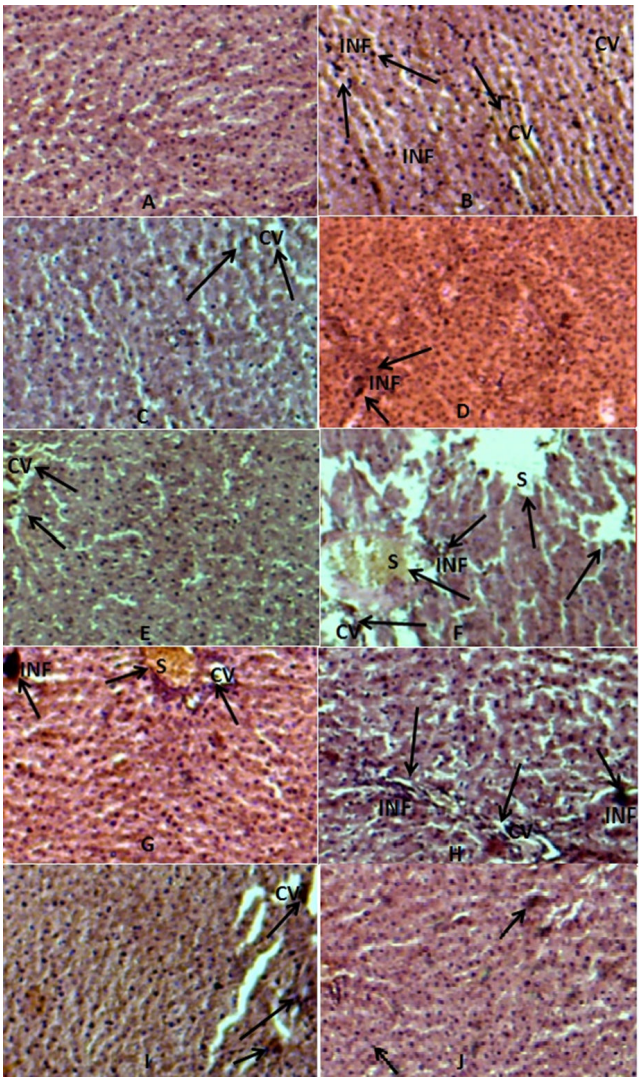

Figure 2: Photographs for histopathology of the liver of rats: A: Control; B: Positive Control, C=4.25 mg/kg Magnesium; D=8.5 mg/kg Magnesium; E=17 mg/kg Magnesium; F=4.25 mg/kg Calcium; G=8.5 mg/kg Calcium; H=17 mg/kg Calcium; I=4.25 mg/kg Magnesium+4.25 mg/kg Calcium; J=4.25 mg/kg Magnesium +8.5 mg/Kg Calcium. CV: Cytoplasmic Vacuolization; INF: Infiltration; S: Steatosis; Magnification X40.

Groups	Cytoplasmic vacuolization	Infiltration of inflammatory cells	Mild to moderate steatosis	Focal hepatocellular necrosis (sporadic)	Nuclear disintegration
Negative control	-	-	-	-	-
Positive control	+	-	-	-	-
4.25 Mg	+		-	-	-
8.5 Mg	-	-	-	-	-
17 Mg			-	-	-
4.25 Ca		-	+	-	-
8.5 Ca		+	+	-	-
17 Ca		+	-	-	-
4.25 Mg +4.25 Ca	+	-	-	-	-
4.25 Mg +8.5 Ca	-	-	-	-	-

Table 4: Histological changes of liver tissue subjected to treatments of alcohol, Maalox plus® and pureCal®. 4.25 Mg=4.25 mg/kg Magnesium; 8.5 Mg=8.5 mg/kg Magnesium; 17 Mg=17 mg/kg Magnesium; 4.25 Ca=4.25 mg/kg Calcium; 8.5 Ca=8.5 mg/kg Calcium; 17 Ca=17 mg/kg Calcium; 4.25 Mg+4.25 Ca=4.25 mg/kg Magnesium+4.25 mg/kg Calcium; 4.25 Mg+8.5 Ca=4.25 mg/kg Magnesium+8.5 mg/kg Calcium.

Discussion

In the first week of the study animals that were treated with alcohol were lethargic, had a rough coat, poor appetite and decreased weight gain. However, from the second week onwards they were more active, had good appetite and recorded significant weight gain. Studies on the effect of alcohol on body weight show mixed results. Cases of significant decrease in body weight gain in rats have been reported and attributed to alcohol altering the storage, excretion and inhibiting breakdown of nutrients [23]. In an earlier study excessive alcohol intake led to rats loosing appetite, being apathetic and having slow responses [37]. Other studies opine to alcohol resulting in positive energy balance and causing weight gain [38-41]. A myriad of factors including gender, genetics, physical activity level, medication use, psychological problems, type, frequency and amount of alcohol intake have been implicated to result in the contradictory evidence on alcohol and body weight [42].

In this study the contradictory effects of alcohol on body weight of rats was demonstrated whereby in week one there was a decrease in weight and this was most likely due to the reduced feed intake and the resultant malnutrition, while from week two onwards the animals developed tolerance to alcohol and the consequent weight gain was attributed to the calorific effects of ethanol.

Animals that were treated with pureCal® calcium had insignificant ($p>0.05$) weight change over four weeks period. These results are consistent with those reported in prior studies where calcium supplementation had no notable effect on body weight [43,44]. Contrary reports of a dose dependent diminutive effect of calcium on body weight in mice exist and this was attributed to a decline in the adipocyte fatty acid synthase activity and an elevation in lipolysis [45]. Calcium supplementation in this study did not seem to have any effect on the synthesis or lysis of adipose tissue hence the insignificant change in body weight of the rats.

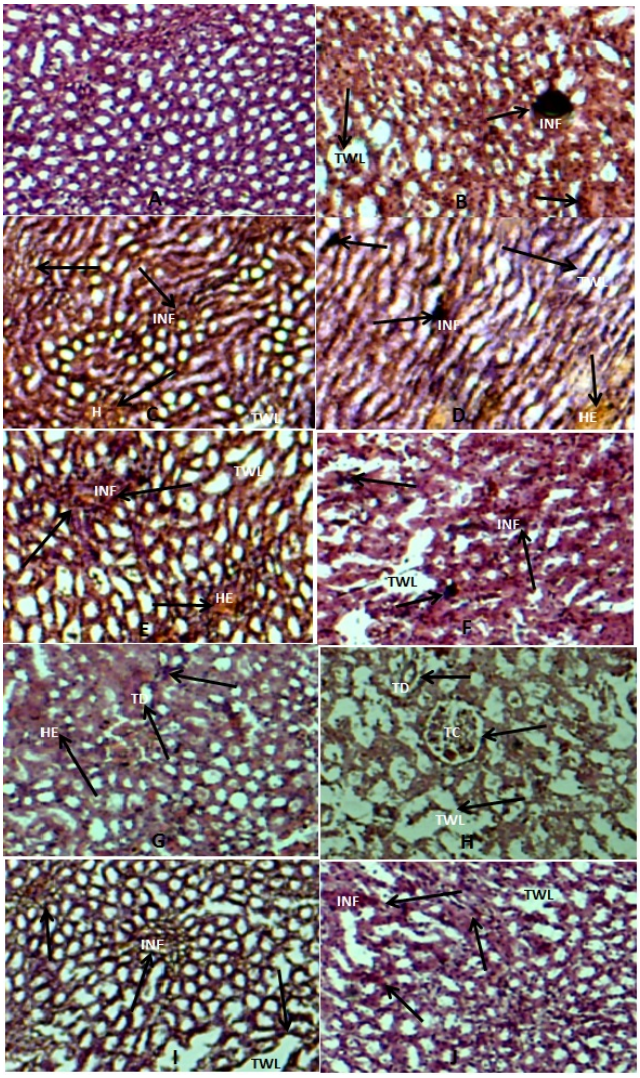

Figure 3: Photographs for histopathology of the kidney of rats. A=Control; B=Positive Control; C=4.25 mg/kg Magnesium; D=8.5 mg/kg Magnesium; E=17 mg/kg Magnesium; F=4.25 mg/kg Calcium; G=8.5 mg/kg Calcium; H=17 mg/kg Calcium; I=4.25 mg/kg Magnesium+4.25 mg/kg Calcium; J=4.25 mg/kg Magnesium +8.5 mg/kg Calcium; TWL: Tubular Widening Lumen; INF: Infiltration; HE: Haemorrhage; TD: Tubular Degeneration; TC: Tubular Cast; Magnification X40.

	Cellular infiltration	Tubular widening lumen	Haemorrhage	Tubular cast	Tubular degeneration	Glomerular shrinkage	Glomerular degeneration
Negative control	-	-	-	-	-	-	-
Positive control	+	+	-	-	-	-	-
4.25 Mg	+	+	+	-	-	-	-
8.5 Mg	+	+	+	-	-	-	-
17 Mg	+	+	+	-	-	-	-
4.25 Ca	+	+	-	-	-	-	-
8.5 Ca	+	+	+	-	-	-	-
17 Ca	+	+	+	+	+	-	-
4.25 Mg+4.25 Ca	+	+	-	-	-	-	-
4.25 Mg+8.5 Ca	+	+	-	-	-	-	-

Table 5: Histological changes of kidney tissue subjected to treatments of alcohol, Maalox plus® and pureCal®. 4.25 Mg=4.25 mg/kg Magnesium; 8.5 Mg=8.5 mg/kg Magnesium; 17 Mg=17 mg/kg Magnesium; 4.25 Ca=4.25 mg/kg Calcium; 8.5 Ca=8.5 mg/kg Calcium; 17 Ca=17 mg/kg Calcium; 4.25 Mg+4.25 Ca=4.25 mg/kg Magnesium+4.25 mg/kg Calcium; 4.25 Mg+8.5 Ca=4.25 mg/kg Magnesium+8.5 mg/kg Calcium.

The effect of Maalox plus® on body weight was inconclusive. Previous studies have associated magnesium deficiency with reduction in body weight [46,47]. This is because of the central role magnesium plays in growth and development [48] especially in the process of cell proliferation and protein synthesis [49].

Alcohol caused significant reduction in the magnesium levels in bones but it had no effects on magnesium levels in muscle and calcium levels in both tissues. Approximately half of total body magnesium is located in soft tissues intracellular with the rest found in bones as surface bound divalent cations that are exchangeable or in the hydroxyapatite. The exchangeable divalent surface bound magnesium cations on the bones act as reservoir for maintenance of extracellular magnesium levels [50]. Magnesium deficiency is a feature found mostly in alcoholic and even in cases where the serum magnesium is normal marked intracellular deficiency is possible [22]. In the current study the serum magnesium levels are not affected in the alcohol ingesting subjects. The significant reduction in the bone magnesium is thus due to the loss of the surface bound magnesium cations due to use in maintenance of serum magnesium levels. The loss of magnesium in bone was effectively reversed through supplementation.

Alcohol is a potent liver toxicant and the severity of the disorder is highly influenced by the concentration of ethanol consumed [51]. In the present study alcohol leads to cellular infiltration and cytoplasmic vacuolization in the liver. Infiltration of neutrophils has been attributed to the apoptosis of hepatocytes, inflammatory mediators, chemokines, cytokines and adhesion molecules [52]. Similar results were reported with antioxidant deficiency as a result of alcohol induced malnutrition being implicated as causative of the damage [30].

Treatment with Maalox plus® and pureCal® in a ratio of 1:2 was found to protect the animals from liver pathology. Potential therapeutic effect of magnesium on the liver damaged due to alcohol toxicity has been documented [22]. It has been pointed out that magnesium enhances the oxidative defence by increasing the activity of antioxidant enzymes thereby strengthening the host's defence by intensifying the anti-oxidative process [30]. Similar findings of an earlier study have seen $MgSO_4$ combating inflammatory responses and oxidative damage that are experienced when suffering from cholestasis liver injury [53]. The ameliorative properties of calcium on hepatotoxicity induced by fluoride on rabbit subjects have also been demonstrated [54], where just like with magnesium, calcium was able to increase the activity of superoxide dismutase, glutathione peroxidase and brought about ultra-structural repair of the liver. It is thus evident that both elements have a curative effect on liver pathophysiology and that it was the 1:2 ratio of Mg:Ca that turned out to be more effective in repairing the liver architecture.

Alcohol was responsible for mild pathology on the kidney in form of cellular infiltration and widening of the tubular lumen. This corroborates prior assertions that the essence of functional disturbances of the kidney after ethanol exposure is down to the ultra-structural abnormalities experienced [55]. Conclusions linking kidney degeneration to alcohol causing a surge in protein and acetaldehyde oxidation and thus causing an increase in reactive oxygen species which are responsible for oxidative stress have also been deduced [51]. Alcohol is known to cause oxidative stress that triggers an inflammatory response responsible for cellular infiltration as was observed in the kidney tissue. Treatment with Maalox plus® and pureCal® was found to have no influence on the cellular infiltration nor the widening of the lumen of the kidney tubules.

Conclusion

Alcohol administration across the groups resulted in the animals being lethargic, having a rough coat, poor appetite and decreased

weight gain. Conditions which improved as time progressed. There was a contradictory effect of alcohol on the body weight of our subjects while supplementation with Maalox plus® gave inconclusive results and pureCal® having no discernible effect.

Findings from tissue analysis showed that alcohol did significantly reduce the bone magnesium levels but it did not affect the bone calcium and the muscle magnesium and calcium levels. Both regimens of Maalox plus® and pureCal® exemplified their therapeutic effect by normalizing the levels of bone magnesium.

Liver tissues from the alcoholic subjects where characterized with cellular infiltration and vacuolization. On the other hand the kidney tissues from the same subjects also displayed cellular infiltration accompanied by widening of the tubular lumen. Though Maalox plus® and pureCal® were not able to alleviate the conditions observed in the kidneys, when administered in a ratio of 1:2 they proved to be hepatoprotective. Administration of pureCal® singly was detrimental as it resulted in steatosis.

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