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The effect of duration of alcohol (ethanol) consumption on calcium, phosphate and alkaline phosphatase levels in albino rat

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Abstract

The present study investigated the effect of duration of alcohol (ethanol) consumption on calcium, phosphate and alkaline phosphatase levels in albino rats. Twenty (20) albino rats were randomly divided into four (4) groups (A-D). Group A received no treatment. Group B, C and D received 30% v/v ethanol (1000mg/kg, oral) daily for one week, two weeks and three weeks respectively. Serum calcium, phosphate and alkaline phosphatase levels were evaluated using standard methods. The results showed a non-significant decrease ($P>0.05$) in serum calcium level after two weeks; which however increased significantly ($P<0.05$) after an additional week of ethanol administration. There was a steady rise in alkaline phosphatase activity from one to three weeks. There was no significant change in serum phosphate level throughout the three weeks of ethanol administration. Acute alcohol consumption may affect the levels of calcium and alkaline phosphatase in the blood but exerts no effect on phosphate level.

Keywords: Alcohol, alkaline phosphatase, calcium, duration of ethanol, phosphate

1. Introduction

Alcohol consumption has become common among the populace. The economic situation and poverty level have made alcohol consumption high among the population; as people tend to resort to high alcohol consumption to give them succor [1]. Alcohol is a drug, classified as a depressant, meaning that it slows down vital functions, resulting in slurred speech, unsteady movement, disturbed perceptions and an inability to react quickly [1]. A positive relation between depression and alcohol consumption has been found [2, 3] and combining antidepressants and alcohol has also become common [3]. There are reports of its interaction with antidepressants in patients [4, 5]. When antidepressants called monoamine oxidase inhibitors (MAOIs) are combined with certain types of alcoholic beverages and foods, a dangerous spike in blood pressure could result. The combination of antidepressants and alcohol affects judgment, coordination, motor skills and reaction time more than alcohol alone [6]. There is no doubt as to the serious medical complications caused by alcoholism. Alcoholism is currently listed as the third leading cause of death in our society [7]. Many alcohol-related deaths go unreported and many health professionals believe that alcoholism is probably the number one killer. As an irritant, alcohol has the potential of causing serious physical harm to the body systems [7]. Acute alcohol abuse causes damage and functional impairment of several organs affecting protein, carbohydrate, and fat metabolism, which results in metabolic disturbance [8]. Alcohol causes three types of liver injury: fatty liver (or steatosis), alcoholic hepatitis and cirrhosis. People who continue abusing alcohol may develop more serious liver damages like fibrosis and even liver failure [9]. Excessive alcohol intake is a well recognized cause of secondary osteoporosis. Alcohol abuse is associated with increase in both the incidence of fracture and complications in fracture healing [10]. Younger alcoholic patients, without other diseases, may suffer from an increased risk to develop low bone mineral density [11]. Higher intakes appear to be associated with an increase fracture risk and hip fracture risk [12]. In a study conducted by Lee *et al.* [13], they revealed that alcohol consumption leads to an elevation of liver enzymes (AST, ALT and ALP). Another study undertaken by Mukamal *et al.* [14], reported a decrease in bone mineral (calcium) and liver triglycerides following alcohol consumption. A further research has to be done to evaluate the effect of duration of alcohol consumption on liver enzyme (alkaline phosphatase) and bone minerals (calcium and phosphate), and to validate previous reports. In addition, there is currently no literature on the

acute impact of alcohol on the bone. Thus, this acute study was designed to evaluate the effect of duration (acute effects) of alcohol consumption on calcium, phosphate and alkaline phosphatase in albino rats.

2. Materials and Methods

2.1 Ethanol

About 2.5 Litre of absolute ethanol was purchase from (Alpha Pharmaceuticals, Enugu, Nigeria). The dose of 1000mg/kg body weight (30% V/V) ethanol was calculated for oral administration.

2.2 Experimental animals and maintenance

Twenty (20) adult male albino rats, with an average weight of (150-200g) were used in this study. They were obtained from the animal house of the College of Medicine, University of Nigeria Teaching Hospital (UNTH) Enugu state, Nigeria. The animals were housed in metallic cage in the animal house under ambient temperature (25±3°C) and 12-hour light and dark periodicity. They were adequately fed with commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and water *ad libitum* and allowed to acclimatize for 2 weeks. Proper care was taken as per the ethical rule and regulation of the concerned committee of the University of Nigeria, Nsukka, Enugu State, Nigeria.

2.3 Experimental design.

A total of 20 male albino rats were used. The rats were randomly allocated to four (4) groups (A–D) of five (5) animals per group in well ventilated cages. The experimental animals received the following treatments on a daily basis for at most three weeks period together with stipulated feed and water.

- Group A (Normal Control) did not receive ethanol.
- Group B received 30% V/V ethanol (1000mg/kg) for 1 week.
- Group C received 30% V/V ethanol (1000mg/kg) for 2 weeks.
- Group D received 30% V/V ethanol (1000mg/kg) for 3 weeks.

2.4 Sacrificing of animal and sample collection

At the end of the experiment, the animals were sacrificed under chloroform anesthesia. Approximately 5ml of blood was obtained via cardiac puncture into plain tube for the determination of calcium, phosphate and alkaline phosphatase levels.

2.5 Biochemical analysis

Serum calcium was determined by direct colorimetric method with O-cresolphthalein complexon as described by Morin [15]. Alkaline Phosphatase (ALP) activity determined by colorimetric method according to Kind and King [16]. Serum phosphate level determined using colorimetric method according to Pesce *et al.* [17].

2.6 Statistical Analysis

The statistical analysis was done using *Graph pad prism6.0*. The results were reported as mean ±SEM (standard error of mean). Statistical significance $p<0.05$ (*), $p<0.01$ (**), or $p<0.001$ (***) was determined by using ANOVA.

3. Results and Discussion

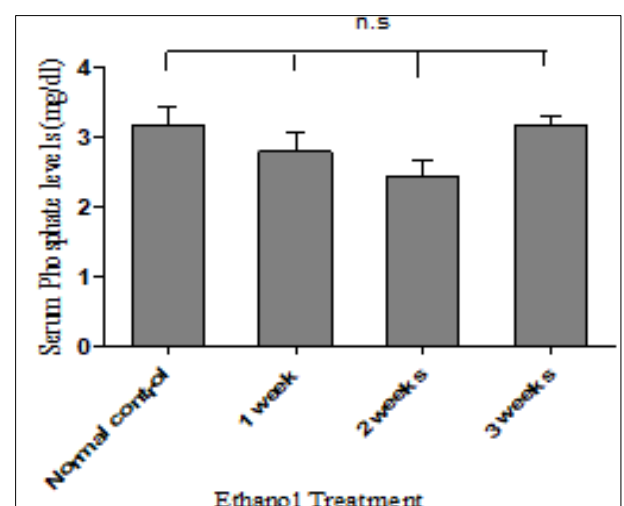
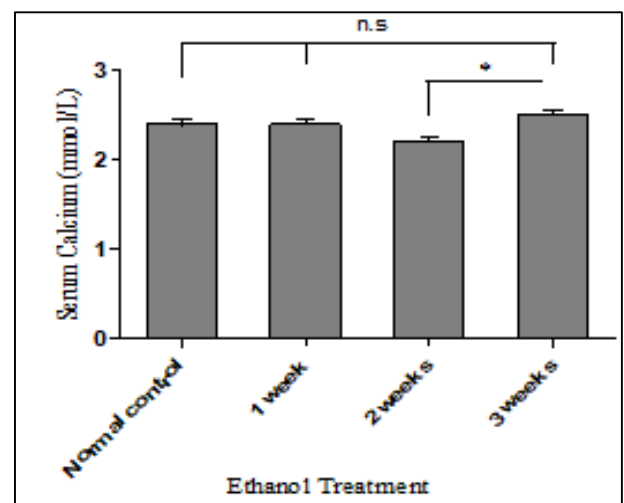
Serum calcium, phosphate and alkaline phosphatase levels in

all groups are shown in table 1. The serum calcium level was non-significantly decreased during 2 weeks of oral administration of 30% v/v ethanol (1000mg/kg). Surprisingly, the calcium levels then showed a significant elevation ($p<0.05$) on continuous administration of the ethanol to 3 weeks. However, there were no significant alterations in serum phosphate levels among the groups irrespective of the duration of administration. In addition, there was steady rise in alkaline phosphate levels with increase in duration of administration. There was significant elevation ($p<0.01$) of ALP levels when normal control is compared with 3 weeks ethanol treatment. [Figure 1]

Table 1: Statistical analysis of serum calcium, phosphates and alkaline phosphatase concentrations in different experimental animal groups.

Treatment Group	Calcium (mmol/L)	Phosphate (mg/dl)	ALP (IU/L)
Normal control	2.40±0.06	3.17±0.26	126.00±10.39**
1 week Ethanol (1000mg/kg)	2.40±0.06	2.80±0.26	292.33±18.66
2 weeks Ethanol (1000mg/kg)	2.20±0.06*	2.43±0.23	305.33±60.24
3 weeks Ethanol (1000mg/kg)	2.50±0.06	3.17±0.15	403.67±61.30

Values are given as Mean ± SEM. ** $P<0.01$ or * $P<0.05$ is significantly different when normal control, 1 week ethanol treatment or 2 weeks ethanol treatment is compared with 3 weeks ethanol treatment.



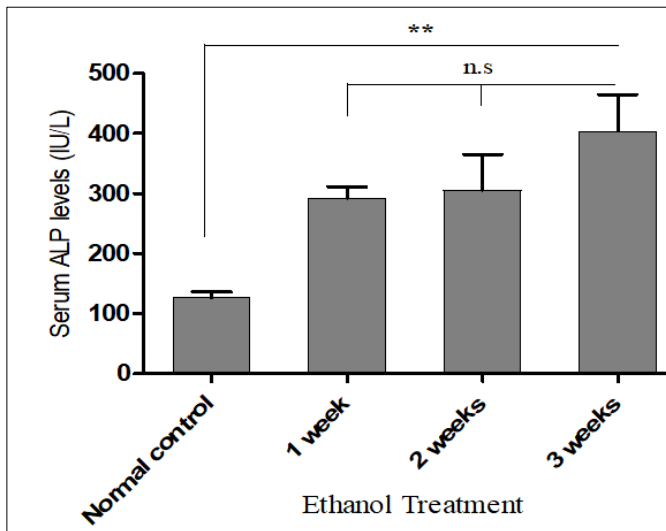


Fig 1: Comparison of serum calcium, phosphates and alkaline phosphatase concentrations in different experimental animal groups.

Histogram showing serum calcium, phosphate and ALP levels following treatment with ethanol for 0 week, 1 week, 2 weeks or 3 weeks. The preliminary data shows that there were only alterations in serum calcium and ALP levels. There was no significant change in phosphate levels. Albino wistar rats ($n = 5$) were daily administered with 30% v/v ethanol (1000mg/kg, oral) for 3 weeks duration. The data are presented as mean \pm SEM of Serum calcium, phosphate and alkaline phosphatase levels for individual treatment. See *Materials and Methods* for experimental details. Statistical analyses were performed using the student's t-test or ANOVA. (** $P < 0.01$; * $P < 0.05$).

Excessive alcohol intake has a deleterious effect on the body system [8]. Osteoporosis, which has been reported as a major cause of fracture in the world today, has been reported to be caused by excessive alcohol intake [11]. Alcoholism causes a change in bone minerals and may lead to the occurrence of fracture complications among humans. The aim of this research was to investigate the effect of duration of alcohol (ethanol) intake on calcium, phosphate and alkaline phosphatase levels in Albino Wistar rats.

The result in table 1 shows that two weeks oral administration of 30% v/v ethanol (1000mg/kg) non-significantly decreased serum calcium level. A further one week oral administration of the ethanol caused a significant elevation in serum calcium level. Furthermore, from the results there was no significant change in phosphate metabolism throughout the three weeks oral administration of the ethanol. In addition, there was a steady rise in alkaline phosphatase levels, with positive relation to increased duration of administration.

Alcohol has an effect on the hormones that control calcium homeostasis (parathyroid hormone, calcitonin and vitamin D) as well as the hormones that indirectly control calcium homeostasis (e.g. steroids reproductive hormones and growth hormone) [18]. Short term alcohol consumption can lead to a temporary parathyroid hormone deficit and increased removal of calcium in the urine resulting in loss of calcium from the body. Long term alcohol intake causes a significant rise in parathyroid hormone release, possibly by removing calcium from the body fluids (e.g. blood) and causing their flow into cells [19]. A brief rise in Calcitonin levels are observed during acute and short term alcohol intake [19]. Low levels of vitamin D are normally present in alcoholics as well as the proteins

that bind with vitamin D to protect it during transport within the blood [20]. Individuals with alcoholic liver disease usually present with low level of Vitamin D (e.g., alcoholic cirrhosis). The low level of vitamin D caused by alcoholism results in decreased absorption of calcium, although the level of calcium following abstinence quickly reverts to normal [21]. The sudden decrease in serum calcium levels after two weeks administration of 30% v/v ethanol (1000mg/kg) may be due to the resultant decrease in parathyroid hormone and increased level of calcitonin secretions in the blood. The increased level of calcitonin prevents osteoclast formation and this leads to deficient calcium release from bone into the blood. A further one week oral administration (i.e. three weeks) of 30% v/v ethanol (1000mg/kg) caused a substantial increase in serum calcium level and this may be as a result of increased parathyroid hormone and decreased calcitonin secretions in the blood. Increased parathyroid hormone secretions causes increased osteoclast formation leading to release of calcium from bone into the blood.

The significantly increased level of alkaline phosphatase activity throughout the three weeks oral administration of 30% V/V ethanol (1000mg/kg) observed in this study maybe due to effect of alcohol on the liver hepatocytes, since serum alkaline phosphatase activity is mainly from the liver with 50% contributed by the bone [10]. This occur as a result of oxidative stress activity of alcohol on liver leading to breakdown of its metabolic products especially acetaldehyde [22]. The oxidative stress induced on the liver by the alcohol causes hepatocytes breakdown which leads to release of the liver enzyme alkaline phosphatase in the blood.

4. Conclusion

Excess alcohol consumption has major harmful effects on bone development and liver hepatocytes. It could be deduced from the study that longer duration of alcohol consumption causes hypercalcaemia and an increased alkaline phosphatase activity in the serum of an individual. Alcohol intake has no acute effect on serum phosphates as was observed.

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