

https://dspace.org/

TestImpact of binge alcohol consumption on mandibular cytoarchitecture and remodeling in adolescent Sprague Dawley rats

Akaashni Bhika; Amadi Ihunwo; Diana Pillay

https://hdl.handle.net/10539/48974
DOAJ

Downloaded from a DSpace repository. https://dspace.org/

CENSA
CENTRO NACIONAL DE SANIDAD AGROPECUARIA

ORIGINAL PAPER

Revista de Salud Animal
Vol. 47, January-December 2025
ISSN: 2224-4700, ISSN-L: 0253-570X
Cu-ID: https://cu-id.com/2248/v47e11

IMPACT OF BINGE ALCOHOL CONSUMPTION ON MANDIBULAR CYTOARCHITECTURE AND REMODELING IN ADOLESCENT SPRAGUE DAWLEY RATS

Impacto del consume excesivo de alcohol en la citoarquitectura y remodelación mandibular en ratas Sprague Dawley adolescentes

Akaashni Bhika[1*], Amadi Ogonda Ihunwo[1], Diana Pillay[1,2]

[1] *Department of Anatomical Sciences, School of Biomedical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.*
[2]*Department of Human Anatomy and Histology, School of Medicine, Sefako Makgatho Health Sciences University, Pretoria, South Africa.*

ABSTRACT: Underage binge drinking is a major concern as it can have a negative impact on the growth and development of the adolescent skeleton, affecting many people worldwide, including in South Africa. Improving our understanding of the effects of binge drinking on the adolescent mandible can help identifying potential intervention measures and raise public awareness about the dangers of alcohol use. The present study was conducted to research how acute binge drinking affected the structure and remodeling of the mandible in adolescent Sprague Dawley rats. The study involved twenty-four Sprague Dawley rats (12 male and 12 female), aged 7 weeks, divided into either an alcohol-exposed group (n = 6 males and 6 females) or a pair-fed control group (n = 6 males and 6 females). The treatment involved administering 3 g/kg of 20 % alcohol three days a week (on alternate days, with a single dose of alcohol administered on these days) for 7 days to the alcohol-exposed groups, while the pair-fed control groups received a caloric equivalent dose of maltose dextrin via oral gavage. Alcohol is high in calories; thus, a pair-fed model was used to control potential weight changes resulting from caloric intake rather than the effects of alcohol itself. Animals were euthanized on day 7 using a pentobarbital injection, and their mandibles were collected and processed for histology and immunohistochemistry staining using Haematoxylin and Eosin, anti-ki67, and anti-alkaline phosphatase (ALP) antibodies. Images were captured using a light microscope equipped with an axiocam HRC digital camera (Zeiss Axioscope 2 plus). Cell quantification was performed using Fiji Image J software where the manual counting method was utilized. The results showed negative changes in the cytoarchitecture in alcohol-exposed groups, and a decrease in osteoblast numbers in the female alcohol-exposed group (female pair-fed control: mean = 136.00 ± 11.81; female alcohol-exposed: mean = 106.00 ± 3.97 (p = 0,008)). There were no significant changes in the number of proliferating cells. Acute binge alcohol exposure negatively affected cytoarchitecture and bone remodeling in the adolescent mandible.

Key words: Adolescence, alcohol, cell-proliferation, cytoarchitecture, immunohistochemistry.

RESUMEN: El consumo excesivo de alcohol en menores de edad es una preocupación importante, ya que puede tener un impacto negativo en el crecimiento y desarrollo del esqueleto adolescente, afectando a muchas personas en todo el mundo, incluyendo a Sudáfrica. Mejorar nuestra comprensión de los efectos del consumo excesivo de alcohol en la mandíbula adolescente puede ayudarnos a identificar posibles medidas de intervención y a concienciar a la población sobre los peligros del consumo de alcohol. El presente estudio se realizó para investigar cómo el consumo excesivo de alcohol agudo afectó la estructura y remodelación de la mandíbula en ratas Sprague Dawley adolescentes. El estudio incluyó veinticuatro ratas Sprague Dawley (12 machos y 12 hembras), de 7 semanas de edad, divididas en un grupo expuesto al alcohol (n = 6 machos y 6 hembras) o un grupo control alimentado por parejas (n = 6 machos y 6 hembras). El tratamiento consistió en administrar 3 g/kg de alcohol al 20 % tres días a la semana (en días alternos, con una dosis única de alcohol administrada en esos días) durante 7 días a los grupos expuestos al alcohol, mientras que los grupos control alimentados por parejas recibieron una dosis calórica equivalente de maltosa dextrina por sonda oral. El alcohol es alto en calorías; por lo tanto, se utilizó un modelo de alimentación por parejas para controlar los posibles cambios de peso derivados de la ingesta calórica, en lugar de los efectos del alcohol en sí. Los animales fueron sacrificados el día 7 mediante una inyección de pentobarbital, y sus mandíbulas fueron recolectadas y procesadas para histología y tinción inmunohistoquímica utilizando anticuerpos de hematoxilina y eosina, anti-ki67 y anti-fosfatasa alcalina (ALP). Las imágenes fueron capturadas utilizando un microscopio óptico equipado con una cámara digital axiocam HRC (Zeiss Axioscope 2 plus). La cuantificación celular se realizó utilizando el software Fiji Image J donde se utilizó el método de conteo manual. Los resultados mostraron cambios negativos en la citoarquitectura en los grupos expuestos al alcohol, y una disminución en los números de osteoblastos en el grupo de hembras expuestas al alcohol (hembra control alimentada por pares: media = 136.00 ± 11.81; hembra expuesta al alcohol: media = 106.00 ± 3.97 (p = 0,008)). No hubo cambios significativos en el número de células proliferantes. La exposición aguda al alcohol afectó negativamente la citoarquitectura y la remodelación ósea en la mandíbula del adolescente.

Palabras clave: Adolescencia, alcohol, proliferación celular, citoarquitectura, inmunohistoquímica.

*Corresponding author: Akaashni Bhika. *E-mail:* akaashni.bhika@wits.ac.za
Received: 25/02/2025
Accepted: 07/04/2025
Conflicts of Interest: The authors declare that they have no competing financial interests or known personal relationships that could have influenced the work presented in this paper.
Author Contributions: Akaashni Bhika: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, visualization, writing - original draft. **Diana Pillay:** conceptualization, methodology, supervision, writing - review and editing. **Amadi Ogonda Ihunwo:** supervision, writing - review and editing. All authors approval of the final version of the manuscript.

This is an open access article under terms of the license Creative Commons Attribution-NonCommercial (BY-NC 4.0).
https://creativecommons.org/licenses/by-nc/4.0/

INTRODUCTION

Alcohol consumption among adolescents in South Africa (SA) and worldwide is a significant public concern due to its harmful impact on adolescent skeletal growth and development (1,2). Previous studies have researched the effects of alcohol on long bones during adolescence, reporting notable reductions in bone growth, volume, density, and strength (3-5). Föger-Samwald *et al*. (2018) examined binge alcohol exposure in prepubescent pigs, finding decreased serum calcium and phosphate levels, along with reduced femoral density and trabecular number (6).

Lauing *et al*. (2008) conducted a study on the effect of binge drinking on adolescent rats, which revealed a significant decrease in cancellous bone mass in the tibia and vertebra after acute binge alcohol exposure. Vertebra showed decreases in bone mass after chronic binge alcohol exposure. Compressive bone strength was also affected (1).

There is limited information on the effect of binge drinking on the adolescent skeleton, especially on the craniofacial structures. The growth and development of the mandible requires control of cell proliferation, differentiation and the ossification processes. Alcohol consumption is known to disrupt these processes (7), thus this study aimed to assess the effect of acute binge drinking on the cytoarchitecture and remodeling of the mandible in adolescent Sprague Dawley (SD) rats.

Enhancing our understanding of the effects of binge drinking on the adolescent mandible can help in identifying the potential effects of adolescent drinking on the mandible, which can have lasting effects on facial aesthetics, implant stability and spacing for dentition. The results of this study can also aid in identifying intervention measures and raising public awareness about the dangers of underage alcohol consumption.

MATERIALS AND METHODS

Ethical clearance

The study began following ethics approval from the Animal Research Ethics Committee at the University of the Witwatersrand (AREC 2020/11/02C). Animal handling and treatment adhered to the committee's established standards and principles.

Animal husbandry

Twenty-four rats (12 males and 12 females) aged seven weeks were placed into either the alcohol-exposed (n = 12) or pair-fed control group (n = 12), at 7 weeks of age weighing approximately 175 g-199 g. All study animals were bred and kept at the University of the Witwatersrand Research Animal Facility (WRAF), Parktown Campus. These animals were maintained under controlled, pathogen-limited conditions, in a temperature-controlled environment (26-28°C) with a 12-hour light/dark cycle. Rats were housed in pairs in plastic cages (2 male rats per cage or 2 female rats per cage) (cage dimensions: 43 mm length, 220 mm width, and 200 mm height), with free movement within the cages. All the animals in the study were fed a standard rodent diet and tap water was provided *ad libitum*.

Treatment with alcohol or maltose dextrin

The animals (n = 24) were placed into either the pair-fed control (n = 12) or the alcohol-exposed group (n = 12) (Fig.1). They were exposed to alcohol for 1 week. Alcohol was administered via oral gavage of a 20 % (vol/vol) alcohol solution (Associated Chemical Enterprises, South Africa) at a dose of 3 g/kg, chosen to achieve peak blood alcohol concentrations (BACs) of approximately 100 mg/dL (8-10). Alcohol was given 3 days/week (on every alternate day as a single dose). No alcohol was administered during the remaining 4 days of the week.

As a caloric equivalent control, there was a pair-fed rat that was matched individually to an alcohol-fed rat based on initial body weight. One gram of alcohol yields 7 kilocalories (kcal), while 1 gram of maltose dextrin yields 3.89 kcal. Consequently, if 1 gram of alcohol equals 7 kcal/g, then a 20 % alcohol solution at a dose of 1.5 g/kg provides 1.4 kcal/g (11). Due to alcohol being calorie dense, a pair-fed model was used to control potential weight changes resulting from caloric intake rather than the effects of alcohol itself. The pair-fed group was given an isocaloric equivalent of maltose dextrin (Sigma-Aldrich, USA) which was also administered via oral gavage. Oral gavage for both groups was performed with a metal, curved, 16-gauge rounded/ bulb-tipped gavage needle.

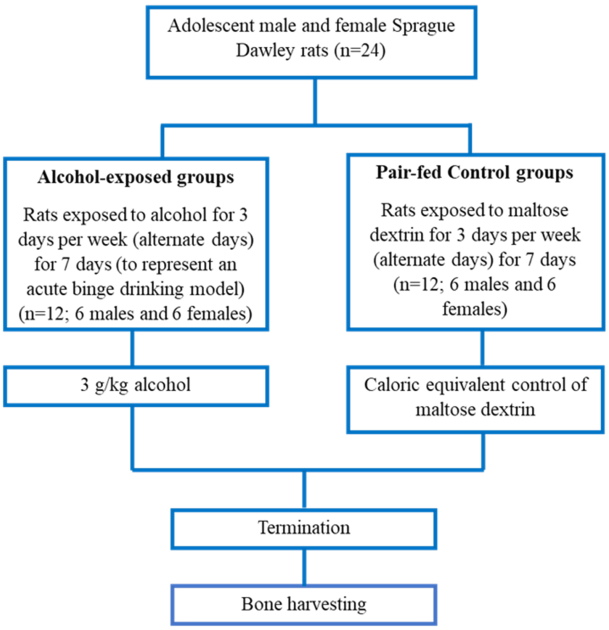


Figure 1. Study design. A diagrammatic illustration of the group allocation of animals in the study. / *Diseño del studio. Ilustración diagramática de la asignación grupal de animales en el estudio.*

Measuring blood alcohol concentration levels

Blood alcohol concentrations (BACs) were tested an hour after exposure by drawing blood using the tail prick method. Blood was stored in heparinized microcapillary tubes (75µl) (Marienfeld, Germany). The estimated blood volume in adult animals is 55 to 70 ml/kg body weight. If repeated blood samples are needed at short intervals, up to 0.6 ml/kg/ day, 1.0 % of the animal's total blood volume can be taken every 24 hours (12). Only 75 µl were drawn per animal, 3 times per week (on exposure days) as this amount was

within the acceptable amount, caused the least possible discomfort to the animals, and was sufficient to perform BAC analysis.

Blood was transferred from the microcapillary tubes into sterile 15 ml conical culture tubes and then centrifuged in a Hettich Rotofix 32A centrifuge (Germany) at 4000 rpm for 10 minutes. Plasma was collected, and its alcohol concentrations were tested using an Alcohol Colorimetric Assay Kit from Sigma-Aldrich (USA), following the manufacturer's instructions. Readings were taken using an iMark Bio-Rad Microplate Absorbance Reader from Bio-Rad Laboratories Inc, USA, at a wavelength of 540nm. All reactions and readings were conducted in an alcohol-free environment.

Skeletal harvesting

The animals were terminated on day 7 by a lethal pentobarbital intraperitoneal injection (200 mg/kg). Skin incisions were made, and mandibles were dissected out carefully, making sure to remove the muscles before removing the bones. Each mandible was then stored individually in 10 % buffered formalin for further fixation and processing for cytoarchitecture and immunohistochemistry analysis.

Tissue processing for histological and immunohistochemical techniques

Mandibles were removed and fixed in 10 % buffered formalin for at least 14 days. The right hemi-mandible was used for Haematoxylin and Eosin (H&E) staining and immunohistochemistry (IHC). Formalin-fixed samples were decalcified in 14 % w/v ethylenediaminetetraacetic acid (EDTA) (pH 7.4) (Sigma-Aldrich, USA) in an incubator with continuous gentle shaking at 45°C for 7 days. Subsequently, the bones were rinsed, and the region of interest was isolated by cutting through the mandible anteriorly, anterior to the 1st molar tooth, and posteriorly, posterior to the third molar tooth. These bone samples were then placed in 10 % buffered formalin 24 hours before processing. The samples were then processed through ascending grades of alcohol overnight using an automatic tissue processor (Citadel 2000, Thermo Fisher Scientific, USA), before embedding in paraffin. During embedding, special care was taken to orient the bone samples longitudinally, with the buccal aspect of the bone-facing the cutting surface of the paraffin block to minimize variations in the sectioning angle. Serial sections were cut with a microtome (Leica RM2125 RTS; Leica Biosystems, Germany) at 5µm thickness and placed on Epredia SuperfrostTM plus adhesion microscope slides (Netherlands). Every third section in the sequence was stained with Hematoxylin and Eosin (H&E) to evaluate mandibular cytoarchitecture or subjected to immunolocalization of the Ki-67 protein (a cell proliferation marker) or anti-alkaline phosphatase (ALP) (an osteoblast maker).

Immunohistochemistry for Ki-67 and ALP

Immunolocalization of mandibular cells expressing the Ki-67 protein and the alkaline phosphatase (ALP) enzyme was performed using the anti-Ki-67 antibody (NB 500-170) (Whitehead Scientific, South Africa) and the anti-ALP antibody (Ab95462) (Abcam, UK) respectively. In summary, the sections were deparaffinized, rehydrated and subsequently immersed in citrate buffer solution (pH=6), kept in a water bath set at a temperature of 60°C for 2 hours to allow for antigen retrieval. Tissue sections were then cooled down to room temperature for 20 minutes before washing for 5 minutes in a phosphate buffer solution (PBS) (pH=7.4). Endogenous peroxidase was blocked with 1 % hydrogen peroxide (H2O2), washed with PBS for 3×5 minutes, incubated with a goat serum protein block (Ab156024) (Abcam, UK) for 20 minutes, and washed in PBS for 3×5 minutes. The tissue sections were then incubated with primary antibody (anti-Ki-67 in 1:200 or anti-ALP in 1:1000 dilution factor) overnight at 4°C. On the second day, sections were allowed to reach room temperature and washed in PBS for 4×5 minutes. A biotinylated secondary antibody (biotinylated goat anti-rabbit IgG [H+L] [ab64256]) (Abcam, UK) was applied for 10 minutes and then washed in PBS for 4×5 minutes. To reduce nonspecific background staining while enhancing antibody binding, sections were incubated with streptavidin horseradish peroxidase for 10 minutes (ab64269) (Abcam, UK) and washed in PBS for 4×5 minutes. For visualization under a light microscope, sections were incubated with 3-3'di-aminobenzidine working solution for 5 minutes and then rinsed in running tap water for 5 minutes. The sections were then dehydrated through alcohol grades, cleared in xylene, and mounted in Entellen.

Photomicrograph acquisition and cell quantification

A light microscope (Zeiss Axioscope 2 plus, Germany) fitted with an axiocam HRC digital camera was used to acquire photomicrographs of the stained slides. These were taken under ×5 (H&E) and ×10 (IHC) magnification. Photomicrographs were transferred into Fiji Image-J software and the region of interest was identified and delineated based on the morphology of the roots of the 1st and 2nd molar teeth. For the quantification of cells, a manual counting method from the images was employed.

Data analysis

The data was managed in Microsoft Excel 365, 2023 (Microsoft Corporation) and analyzed using the Statistical Package for Social Service (SPSS®) version 28 (IBM Co.). The descriptive data of the number of chondrocytes and quantity of Ki-67 and ALP immunopositive cells were reported as means and standard deviation. The Shapiro-Wilk test was used to assess the normality of the data. The Independent Samples t-test was used to analyze normally distributed data, comparing means between the pair-fed control and alcohol-exposed groups. Non-parametric data was analyzed using the Mann-Whitney U test. The threshold for statistical significance was set at $p < 0,05$.

RESULTS

Blood alcohol concentration

Mean blood alcohol concentration (BAC) was 108.04 mg/dL (± 16.60) in the alcohol group, whereas the pair-fed control had negligible amounts.

Mandibular histology and immunohistochemistry

Cell morphology of the mandible

The cytoarchitecture between the roots of the 1st and 2nd molar teeth of the mandible are shown in Figure 2. Pair-fed control rats (Fig. 3A and C) exhibited abundant osteocytes scattered evenly throughout the tissue. Abundant osteoblasts were also detected on the inner surface of trabeculae. Numerous small trabecular spaces were exhibited throughout the tissue and were filled with erythrocytes. A widespread bone matrix was identified. Bone matrix, collagen fibres and periodontal ligament fibres were exhibited surrounding the roots of the teeth. Bone still appeared less mineralized in some areas with a wavy somewhat irregular appearance of collagen fibres in the bone matrix, with a large cell number (osteocytes and osteoblasts). Widespread small blood vessels were exhibited throughout the tissue. The cytoarchitecture of the alcohol-exposed groups (Fig. 3B and D) showed the same pattern as the pair-fed control group except for a few differences.

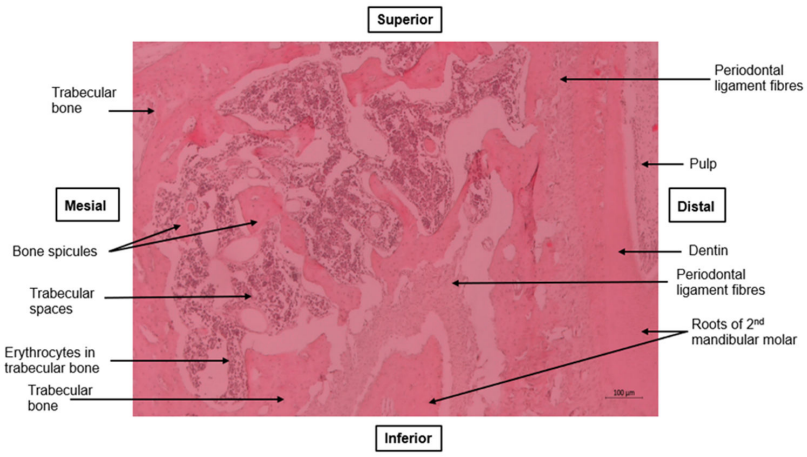


Figure 2. A photomicrograph showing a coronal section of the area between the roots of the 1st and 2nd molar teeth of the mandible (taken from the buccal aspect of the bone). Scale bar = 100 microns at x 5 magnification. For orientation purposes. / *Microfotografía que muestra una sección coronal del área entre las raíces del primer y segundo molar de la mandíbula (tomado del aspecto bucal del hueso). Barra de escala = 100 micras con aumento de x 5. Para fines de orientación.*

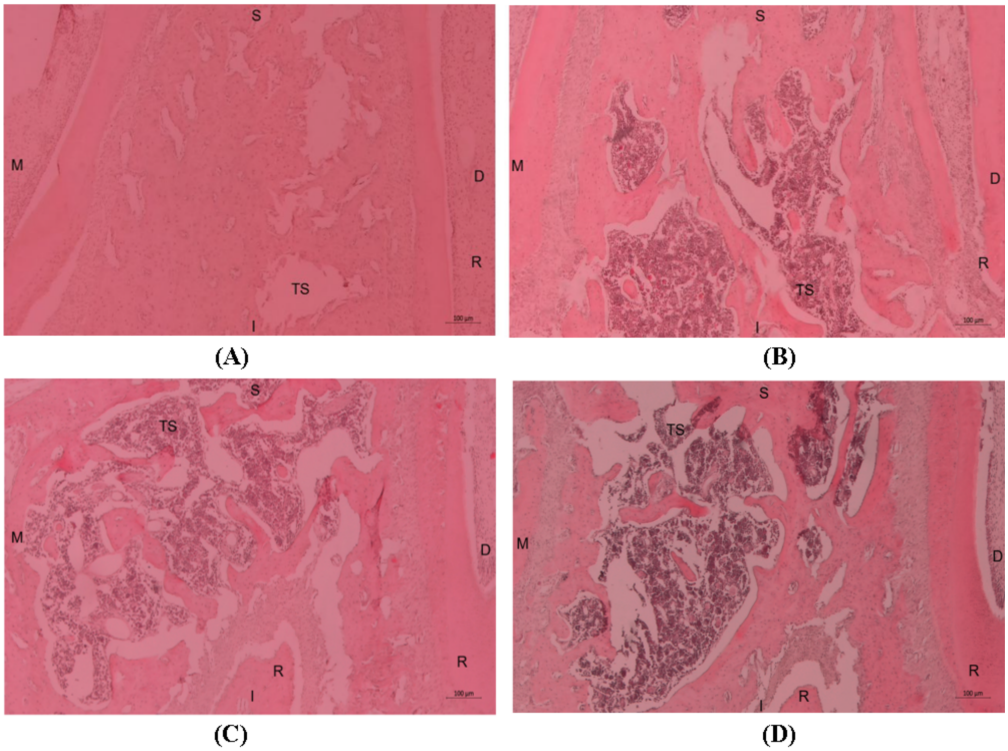


Figure 3. Representative sections of the mandible between the roots of the 1st and 2nd molar teeth of the mandible in an acute binge alcohol model. (A) male pair-fed control group; (B) male alcohol-exposed group; (C) female pair-fed control group; (D) female alcohol-exposed group. H & E staining**.** M=mesial; D=distal; S=superior; I=inferior; R=root; TS=trabecular space. Scale bar = 100 microns at x 5 magnification. / *Secciones representativas de la mandíbula entre las raíces del primer y segundo molar en un modelo de consumo excesivo de alcohol. (A) Grupo control de hombres alimentados en pareja; (B) Grupo de hombres expuestos al alcohol; (C) Grupo control de mujeres alimentadas en pareja; (D) Grupo de mujeres expuestas al alcohol. Tinción con hematoxilina y eosina. M = mesial; D = distal; S = superior; I = inferior; R = raíz; TS = espacio trabecular. Escala de 100 micras con 5 aumentos.*

Trabecular spaces in this group were slightly larger than those exhibited in the pair-fed control group. Bone in this group also appeared less mineralized when compared to the pair-fed control group as collagen fibres were less organized.

Male pair-fed control and alcohol-exposed groups had smaller trabecular spaces than the female-pair-fed control and alcohol-exposed groups and lacked the small islands/spicules of osteoid found in between the erythrocytes in these trabecular spaces. Male pair-fed control and alcohol-exposed groups contained smaller blood vessels and slightly more organised collagen fibres in the bone matrix compared to the female groups.

Ki-67 and alkaline phosphatase immuno-positive cells

There were no significant differences shown between the male pair-fed control (mean = 156.00 ± 8.46) and the alcohol-exposed group (mean = 160.00 ± 2 1.87) (p = 0,409). Fewer proliferating cells were exhibited between the female pair-fed control group (mean = 129.00 ± 10.40) (Fig.4A and Fig.6) and the female alcohol-exposed group (mean = 141.00 ± 17.62) (Fig.4B and Fig.6). This difference was not statistically significant (p = 0,165). A significant difference in the number of proliferating cells was exhibited between the male and female pair-fed control groups (p = 0,011), where the male pair-fed control rats had more proliferating cells than the female pair-fed control rats. The same pattern was detected between the male and female alcohol-exposed rats; however, this difference was not significant (p = 0,160) (Fig.6).

The number of osteoblasts in the male pair-fed control group (mean = 153.00 ± 13.04) (Fig 5A) did not differ from that exhibited in the male alcohol-exposed group (mean = 152.00 ± 17.54) (p = 0,461) (Fig.5B). Female pair-fed control animals (mean = 136.00 ± 11.81) (Fig.5C and Fig.7) had a significantly greater number of osteoblasts than the alcohol-exposed group (mean = 106.00 ± 3.97) (p = 0,008) (Fig.5D and Fig.7). No significant differences were noted in the number of osteoblasts in the male pair-fed control *vs.* female pair-fed control groups (p = 0,085). Male alcohol-exposed rats had significantly greater amounts of osteoblasts than female alcohol-exposed rats (p = 0,006) (Fig.7).

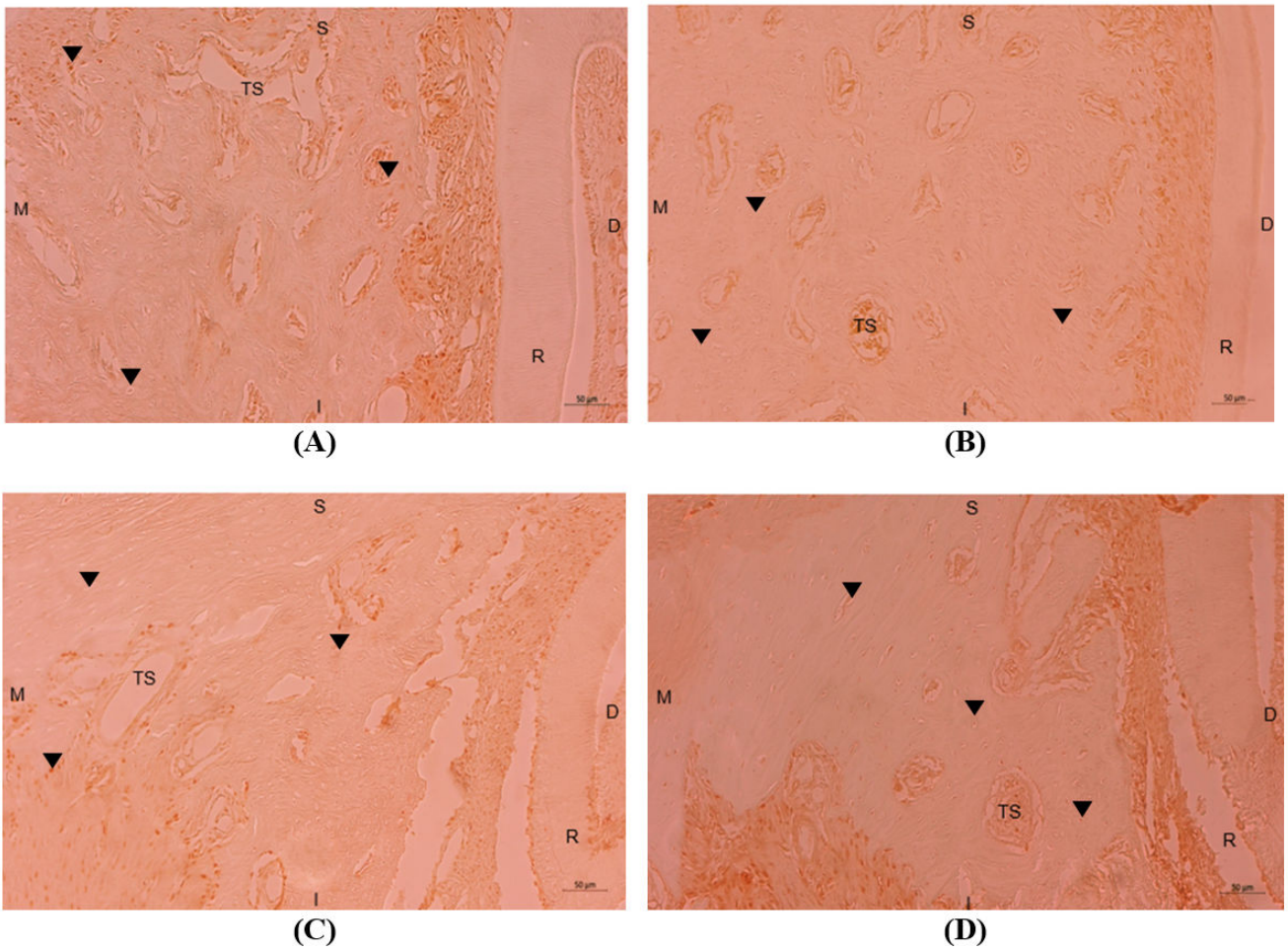


Figure 4. Representative photomicrographs of Ki-67 immunolabeled sections of the mandible between the roots of the 1st and 2nd molar teeth of the mandible in the acute binge alcohol model. (a) male pair-fed control; (b) male alcohol-exposed; (c) female pair-fed control; (d) female alcohol-exposed. Scale bar = 50 microns at x 10 magnification. M=mesial; D=distal; S=superior; I=inferior; R=root; TS=trabecular space. Black arrowheads indicate proliferating cells. / *Fotomicrografías representativas de secciones inmunomarcadas con Ki-67 de la mandíbula entre las raíces del primer y segundo molar en el modelo de consumo excesivo de alcohol agudo. (a) Hombre control alimentado por parejas; (b) Hombre expuesto al alcohol; (c) Mujer control alimentada por parejas; (d) Mujer expuesta al alcohol*. Escala de 50 micras a 10 aumentos. M = mesial; D = distal; S = superior; I = inferior; R = raíz; TS = espacio trabecular. Las flechas negras indican células proliferantes.

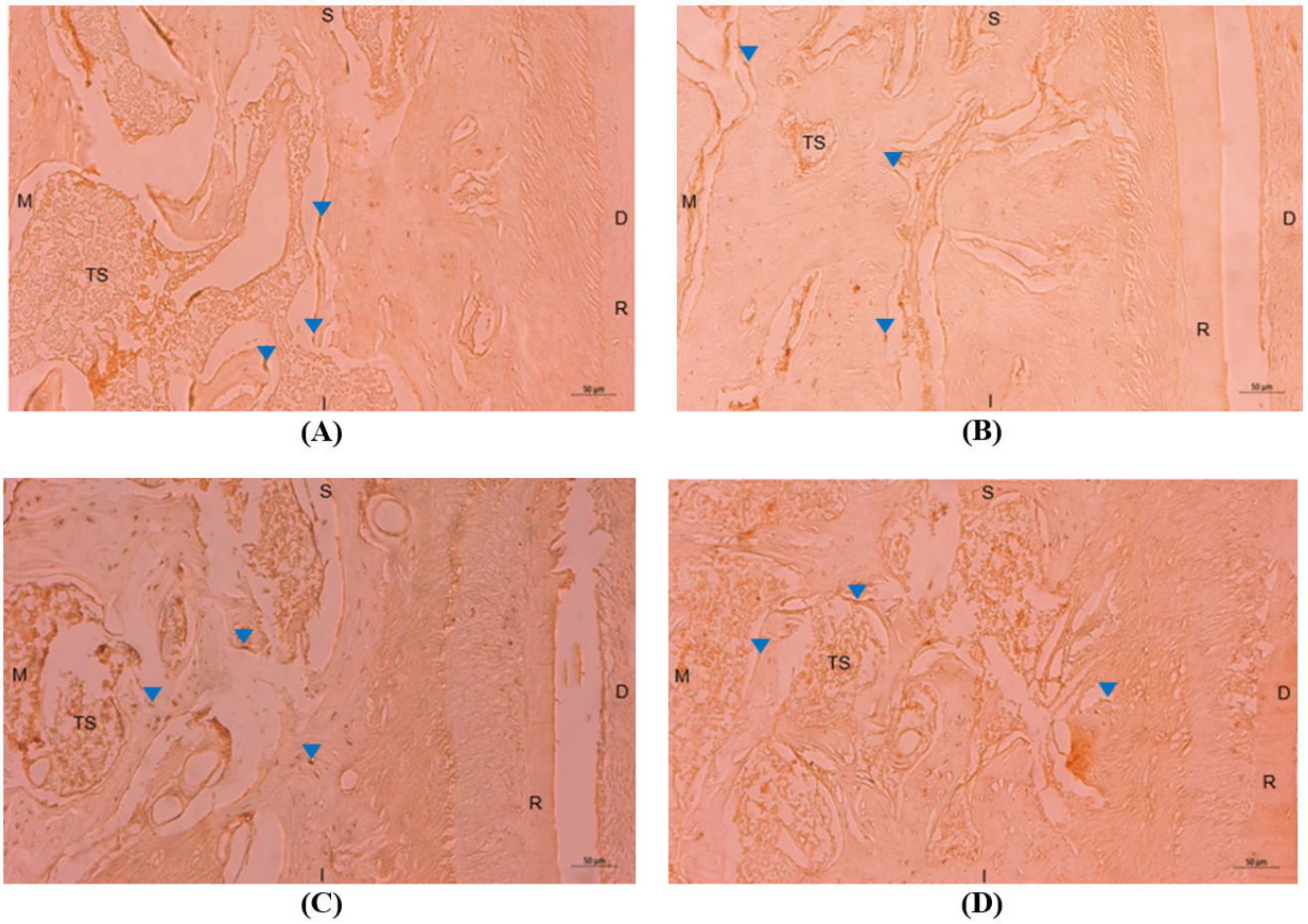


Figure 5. Representative photomicrographs of ALP immunolabeled sections of the mandible between the roots of the 1st and 2nd molar teeth of the mandible in the acute binge alcohol model. (a) male pair-fed control; (b) male alcohol-exposed; (c) female pair-fed control; (d) female alcohol-exposed. Scale bar = 50 microns at x 10 magnification. M=mesial; D=distal; S=superior; I=inferior; R=root; TS=trabecular space. Blue arrowheads indicate osteoblasts (on the surface of the bone) and osteogenic cells. / *Fotomicrografías representativas de secciones de la mandíbula inmunomarcadas con fosfatasa alcalina (FA) entre las raíces del primer y segundo molar en el modelo de consumo excesivo de alcohol agudo. (a) Hombre control alimentado por parejas; (b) Hombre expuesto al alcohol; (c) Mujer control alimentado por parejas; (d) Mujer expuesta al alcohol. Escala de 50 micras a 10 aumentos. M = mesial; D = distal; S = superior; I = inferior; R = raíz; TS = espacio trabecular. Las puntas de flecha azules indican osteoblastos (en la superficie ósea) y células osteogénicas*.

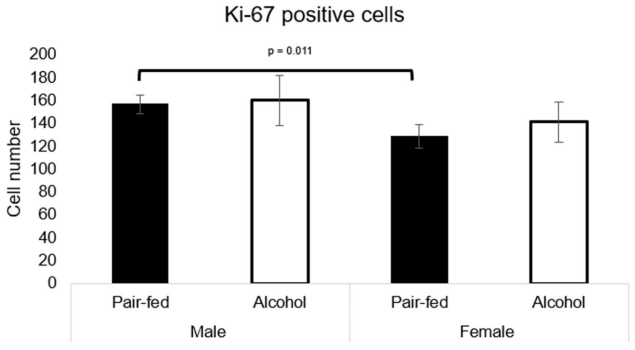


Figure 6. Ki-67 immuno-positive proliferative cells of the mandible in the acute binge alcohol model. The mean number of Ki-67 immuno-positive proliferative cells are shown for the region between the 1st and 2nd molar teeth in the pair-fed control and alcohol-exposed groups in both male and female rats. Error bars indicate standard deviation. / *Células proliferativas inmunopositivas para Ki-67 de la mandíbula en el modelo de consumo excesivo de alcohol. Se muestra la media de células proliferativas inmunopositivas para Ki-67 en la región entre el primer y el segundo molar en los grupos control alimentados por pares y expuestos al alcohol, tanto en ratas macho como hembra. Las barras de error indican la desviación estándar*.

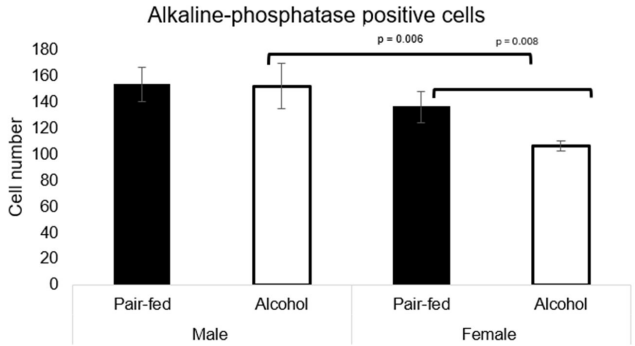


Figure 7. Alkaline phosphatase (ALP) immuno-positive osteogenic cells of the mandible in the acute binge alcohol model. The mean number of osteoblasts is shown for the region between the 1st and 2nd molar teeth in the pair-fed control and alcohol-exposed groups in both male and female rats. Error bars indicate standard deviation. / *Células osteogénicas inmunopositivas para fosfatasa alcalina (FA) de la mandíbula en el modelo de consumo excesivo de alcohol. Se muestra el número medio de osteoblastos en la región entre el primer y el segundo molar en los grupos control alimentados por pares y expuestos al alcohol, tanto en ratas macho como hembra. Las barras de error indican la desviación estándar*.

DISCUSSION

In this study, it was first determined whether acute binge alcohol consumption had any impact on the cytoarchitecture of the adolescent mandible. Second, the primary objective was to establish whether proliferating cells in the adolescent mandible were generally affected and, more specifically, whether this drinking model had any effect on osteoblasts in the adolescent mandible. This study used pair-fed control and alcohol-exposed male and female Sprague Dawley rats that were exposed to maltose dextrin and alcohol respectively via oral gavage. Previous studies suggest that oral gavage induces stress in laboratory animals which may have physiological effects (13).

The cytoarchitecture of the alcohol-exposed animals showed larger trabecular spaces than the pair-fed control animals. Smaller trabecular spaces are detected in more mature bone, possibly indicating that alcohol played a role in delaying bone formation in alcohol-exposed animals (14). No information was available in the literature to confirm this suggestion. The bone matrix in the alcohol-exposed groups also showed less mineralization than the bone matrix exhibited in the pair-fed control groups, as the alcohol-exposed groups displayed more prominent, disorganized collagen fibres. Alcohol can affect the skeleton by altering mineral homeostasis and can directly affect the bone cells. Alcohol can also decrease the mineralization rate and the synthesis rate of the bone matrix (15), which is likely what occurred in the present study in the alcohol-exposed animals. Turner *et al*., 1987 found that alcohol had a negative effect on the histomorphometry of alcohol exposes rats (15). The results of the current study indicate that acute binge alcohol exposure had a negative effect on bone formation and mineralization.

The alcohol-exposed groups did not show any differences in cell numbers when compared to the pair-fed control group. This is contrary to what Miralles-Flores and Delgado-Baeza (1992) and Pillay and Ndou (2024) reported in their studies, where they found that alcohol caused a decrease in the number of proliferating cells in the growth plate of the hypertrophic zone in rats after prenatal alcohol exposure (16,17).

No studies can be found in the literature that report on Ki-67 immuno-positive proliferative cells in the mandible regarding alcohol exposure. Proliferating cells in an adolescent study indicate active growth. A lack of differences between the alcohol-exposed and pair-fed animals could be due to the short duration of the acute binge consumption model.

A decrease in the number of osteoblasts was displayed in the female rats exposed to alcohol in this study. This was corroborated with previous studies which suggest that alcohol consumption can be associated with osteopenia which is likely due to a decrease in osteoblast activity (18-20). Dyer *et al*. (1998) also identified a decrease in the histology of bone formation in three-month-old female Sprague Dawley rats after 6 weeks of alcohol exposure, which they attributed to the suppression of both osteoblast number and function by alcohol (21).

Both the number of proliferating cells and osteoblasts were higher in the male groups compared to the female groups. This is likely due to sexual dimorphism, as males have larger and more robust bones (22,23), thus it is plausible that they would also have more proliferating cells and osteoblasts. The results of the current study indicate that acute binge alcohol exposure did not influence the proliferating cells; however, it did have a negative effect on the number of osteoblasts present in the mandible, corresponding with the decrease in bone formation and mineralization observed in the cytoarchitecture.

In conclusion, acute binge alcohol consumption in adolescents affects osseous tissue by disturbing the cytoarchitecture of the mandible leading to a delay in maturation and mineralization of the bone. Acute alcohol exposure also caused a decrease in osteoblasts and thus had an adverse impact on bone formation. The authors of this study suggest that this may provide some insight into the effect of acute binge alcohol consumption on the adolescent mandible. Additionally, this study demonstrates that even brief binge drinking, such as three days of exposure, causes negative effects on the growth and development of the developing mandible. Communities need to be informed about the poor outcomes of consuming alcohol during adolescence.

ACKNOWLEDGEMENTS

Ms. Hasiena Ali provided technical assistance, Mr Ronald Mngoma and Dr. Nuro Bello assisted with the animal study, and the staff of the University of the Witwatersrand Research Animal Facility (WRAF) assisted with animal husbandry.

REFERENCES

1. Lauing K, Himes R, Rachwalski M, Strotman P, Callaci JJ. Binge alcohol treatment of adolescent rats followed by alcohol abstinence is associated with site-specific differences in bone loss and incomplete recovery of bone mass and strength. Alcohol. 2008;42(8):649-656.
2. Morojele NK, Ramsoomar L. Addressing adolescent alcohol use in south Africa. S Afr Med J. 2016;106(6):551-553.
3. Mngoma NR, Bhika A, Pillay D. The Effects of Acute Binge Alcohol Consumption on the Trabecular Morphometry and Tensile Strength of Adolescent Sprague Dawley Rat Femora. Int J Morphol. 2024;42(2):452-457.
4. Ferreira R, Fernandes R, Bittencourt LO, Alvarenga MO, Cartágenes S, Fernandes L, Maia C, Lima R. Bone impairment in adolescent female rats chronically exposed to ethanol. Indian J Exp Biol. 2022;60(4):258-62.
5. Sampson HW. Alcohol, osteoporosis, and bone regulating hormones. Alcohol Clin Exp Res. 1997;21(3):400-403.
6. Föger-Samwald U, Knecht C, Stimpfl T, Szekeres T, Kerschan-Schindl K, Mikosch P, Pietschmann P, Sipos W. Bone effects of binge alcohol drinking using prepubescent pigs as a model. Alcohol Clin Exp Res. 2018;42(11):2123-2135.
7. Thobane N, Ndou R, Pillay D. The Impact of Gestational Alcohol on the Trabecular Bone Structure of the Mandible in 3-Week-Old Sprague Dawley Rats. Int J Morphol. 2024;42(5):1200-1207.
8. Bhika A, Pillay D, Ihunwo AO. Effect of a Dose-Dependent Administration of Binge Alcohol on the Mandible in the Adolescent S prague Dawley Rat. Int J Morphol. 2024;1(2):607-613.

9. Jeanblanc J, Rolland B, Gierski F, Martinetti MP, Naassila M. Animal models of binge drinking, current challenges to improve face validity. Neurosci Biobehav Rev. 2019;106:112-21.
10. Walker BM, Ehlers CL. Age-related differences in the blood alcohol levels of Wistar rats. Pharmacol Biochem Behav. 2009;91(4):560-5.
11. Bertola A, Mathews S, Ki SH, Wang H, Gao B. Mouse model of chronic and binge ethanol feeding (the niaaa model). Nat Protoc. 2013;8(3):627-637.
12. Parasuraman S, Raveendran R. Biological sample collection from experimental animals. Introduction to Basics of Pharmacology and Toxicology: Volume 3: Experimental Pharmacology: Research Methodology and Biostatistics. 2022;16:45-63. Singapore: Springer Nature Singapore.
13. Teixeira-Santos L, Albino-Teixeira A, Pinho D. An alternative method for oral drug administration by voluntary intake in male and female mice. Lab Anim. 2021;55(1):76-80.
14 Making and shaping endochondral and intramembranous bones. Dev Dyn. 2021;250(3):414-49.
15. Turner RT, Greene VS, Bell NH. Demonstration that ethanol inhibits bone matrix synthesis and mineralization in the rat. J Bone Miner Res. 1987;2(1):61-6.
16. Miralles-Flores C, Delgado-Baeza E. Histomorphometric analysis of the epiphyseal growth plate in rats after prenatal alcohol exposure. J Orthop Res. 1992;10(3):325-36.
17. Pillay D, Perry V, Ndou R. Alcohol intake during pregnancy reduces offspring bone epiphyseal growth plate chondrocyte proliferation through transforming growth factor β-1 inhibition in the Sprague Dawley rat humerus. Anat Cell Biol. 2024;57(3):400-407.
18. Wang X, Chen X, Lu L, Yu X. Alcoholism and osteoimmunology. Curr Med Chem 2021; 28(9):1815-28.
19. Eby JM, Sharieh F, Callaci JJ. Impact of alcohol on bone health, homeostasis, and fracture repair. Curr Pathobiol Rep. 2020;8:75-86.
20. Turner RT. Skeletal response to alcohol. Alcoholism: Alcohol Clin Exp Res. 2000;24(11):1693-701.
21. Dyer SA, Buckendahl P, Sampson HW. Alcohol consumption inhibits osteoblastic cell proliferation and activity in vivo. Alcohol. 1998;16(4):337-41.
22. Gordon and Gordon Gordon RJ, Gordon CM. Adolescents and bone health. Clin Obstet Gynecol. 2020;63(3):504-11.
23. Corona G, Vena W, Pizzocaro A, Giagulli VA, Francomano D, Rastrelli G, Mazziotti G, Aversa A, Isidori AM, Pivonello R, Vignozzi L. Testosterone supplementation and bone parameters: a systematic review and meta-analysis study. J Endocrinol Invest. 2022;45(5):911-926.