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Effect of a Dose-Dependent Administration of Binge Alcohol on the Mandible in the Adolescent Sprague Dawley Rat

Efecto de una Administración Dosis-Dependiente de Alcohol en Exceso en la Mandíbula de la Rata Sprague Dawley Adolescente

Akaashni Bhika[1]; Diana Pillay[2] & Amadi Ogonda Ihunwo[1]

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SUMMARY: Binge drinking in adolescents has a negative effect on the developing skeleton and the attainment of peak bone mass. Our study aimed to examine the effect of binge drinking on the growth and functional integrity of the adolescent Sprague Dawley rat mandible and to determine if a dosage of 1.5 g/kg is sufficient to produce a binge-model of consumption. A total of eight 7-week-old adolescent (male) Sprague Dawley rats were randomly placed into 4 groups with two rats each: 1-week alcohol-exposed rats, 1-week pair-fed control rats, 4-week alcohol-exposed rats and 4-week pair-fed control rats. The alcohol exposed groups were administered a single daily dose via oral gavage of 1.5 g/kg of 20 % alcohol 3 days a week (alternate days) for 7 or 28 days. The pair-fed control groups were administered a caloric equivalent dose of maltose dextrin via oral gavage on the same days as the alcohol-exposed rats. The one-week alcohol exposed, and control rats were terminated on day 7 and the four-week alcohol exposed and control rats on day 28. The mandibles were dissected out and osteometric measurements determined using a digital vernier caliper. Bones were scanned using a 3D-microCT scanner (Nikon XTH 255L). Biomechanical tests were done using a Shimadzu universal testing machine. Differences observed were regarding mandibular osteometry, which showed a reduced height in the central portion of the alveolar bone (Al'-Me), and an increase in the height of the condylar head (Cd-Ag) in the 1-week alcohol-exposed rats when compared to the 1-week pair-fed control rats. No other differences were noted. Lack of significant changes seen between the alcohol and pair-fed control groups in both acute binge and chronic binge exposed rats is likely due to the low dose of alcohol administered to the rats in the study thus a higher dose is proposed.

KEY WORDS: Binge alcohol, Skeletal growth, Osteometry, Biomechanical test, Adolescent mandible.

INTRODUCTION

Alcohol consumption in adolescents in South Africa (SA) constitutes a significant public concern. The drinking pattern of this age group is commonly binge or heavy episodic drinking (Morojele & Ramsoomar, 2016). Binge drinking is the act of consuming ≥5 drinks for males and ≥4 drinks for females on one occasion or during one session of drinking, or consuming ≥ 60 g of pure alcohol at least once per month (Morojele & Ramsoomar, 2016). Pharmacologically relevant blood alcohol concentrations (BACs) of approximately 1 g/l or (100 mg/dl) need to be achieved in a rodent binge drinking model, as rodents metabolize alcohol much more rapidly than humans. Thus, administering a dose of 1.5 g/kg yields a BAC of ±100 mg/dl after 1 hour of administration (Walker & Ehlers, 2009; Jeanblanc *et al*., 2019). It has been reported that binge drinking results in a BAC of 80 mg/dl or above (Föger-Samwald *et al*., 2018).

Adolescence is a period of rapid skeletal growth and development that takes place between the ages of 10 and 19 years. Approximately 90 % of skeletal development and attainment of peak bone mass occurs during this stage of life. A deficiency of peak bone mass significantly contributes to an increased risk of osteoporosis later in life (Weaver, 2002).

The effects of binge drinking on bone growth is still unclear, warranting further investigation. Lauing *et al*. (2008), reported on the effect of binge drinking on adolescent rats. The results of the study showed a significant decrease in cancellous bone mass in the tibia and vertebra after acute binge exposure. Vertebra showed decreases in bone mass after chronic binge exposure. Compressive bone strength was also affected (Lauing *et al*., 2008). A study by Föger-Samwald *et al*. (2018) investigated the effects of alcohol on

[1] School of Anatomical 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, Ga-Rankuwa, South Africa.
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a prepubescent pig model. Their study found fewer trabeculae in the femur (Föger-Samwald *et al*., 2018).

There is a dearth of information regarding the effect of binge drinking on the adolescent skeleton and more specifically on the craniofacial structures. Furthermore, research findings on the effects of alcohol on the adolescent mandible is scarce and studies in this niche area is warranted since the mandible is important for facial aesthetics, implant stability and spacing for dentition.

MATERIAL AND METHOD

Study animals: The research protocol with animal experimentation was approved by the Animal Research Ethics Committee, University of the Witwatersrand (AREC 2020/11/02C). Every effort was made to minimize suffering. A total of 8 adolescent (male) Sprague Dawley rats at 7 weeks (49 days) of age and weighing approximately 175 g-199 g were used. The equivalence of 7-week (49) day old rats in human years is 14.5 human years (Sengupta, 2013). All study animals were bred and kept at the University of Witwatersrand Research Animal Facility (WRAF), Parktown Campus. These animals were maintained under controlled conditions that were free of most pathogens, in a temperature-controlled environment (26-28 °C ±2 °C) and a 12-hour light/dark cycle. The rats were housed in plastic cages of 43 mm long, 220 mm wide and 200 mm height with free movement within the cages. They were housed in pairs. All the animals in the study were fed the standard rodent diet and water was provided *ad libitum.*

Group Allocation

Acute binge model

1-week alcohol-exposed rats. The animals were exposed to alcohol for 1 week. Alcohol was administered by a single daily dose via oral gavage of a 20 % (vol/vol) alcohol solution at a dose of 1.5 g/kg, chosen to achieve peak blood alcohol concentrations (BACs) of approximately 100 mg/dL (Walker & Ehlers, 2009; Jeanblanc *et al*., 2019). BACs were tested an hour after exposure by drawing blood using the tail prick method. Blood was stored in heparinized microcapillary tubes (Marienfeld). BACs were tested using an alcohol colorimetric assay kit (Sigma-Aldrich) and readings were done using an absorbance reader at a wavelength of 630 nm. Alcohol was given 3 days/week (on every alternate day). No alcohol was administered during the remaining 4 days of each week.

1-week pair-fed control rats. 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. The pair-fed group was given an isocaloric equivalent of maltose dextrin (Sigma-Aldrich) which was also administered via oral gavage.

Chronic binge model

4-week alcohol-exposed rats. These animals were exposed to alcohol for 4 weeks. Alcohol was administered as per the 1-week alcohol exposed rats (Lauing *et al*., 2008).

4-week pair-fed control rats. These animals were given maltose dextrin for 4 weeks as per the 1-week pair-fed control rats.

The animals were terminated on day 7 (acute binge model), and day 28 (chronic binge model) by a lethal pentobarbital intraperitoneal injection. The mandibles were then dissected out and stored in 10 % buffered formalin for further fixation and processing.

Osteometric measurements. A digital vernier calliper was used to record the following osteometric measurements, which were an estimation of general mandibular size (Fig. 1, Table I).

Trabecular morphometry. A Nikon XTH 225/320 LC X-ray microtomograph was used for computed tomography (3D-μCT) using the following parameters: X-ray voltage of 100 kv, X-ray current of 100 ma, a scanning resolution of 15 μm, and a scan duration of 18 minutes. VG studio Max® 3.5 software was used for analysis of the following parameters: trabecular thickness (Tb/Th), number (TbN),

Table I. Measurements for estimation of general mandibular size (Adapted from Maki *et al*., 2002).

Measurement	Description
MF-MA	Distance between Mental foramen (MF) and the deepest point of the outer margin of bone which connects Me and Ag
Cd-Id	Length of the base of the mandible which is the distance between the condyle to Infradentale (labial side)
Cd-Ag	Height of condylar head which is the distance between the Condyle (Cd) and the Ante-gonion (Ag)
Cr- Ag	Height of coronoid which is the distance between the Coronoid (Cr) and Ante-gonion (Ag)
Al´-Me	Height of the central portion of alveolar bone which is the distance between the deepest point of the outer margin of bone (Al´) that connects Menton (Me) and the Ante-gonion (Ag) to the Menton (Me)

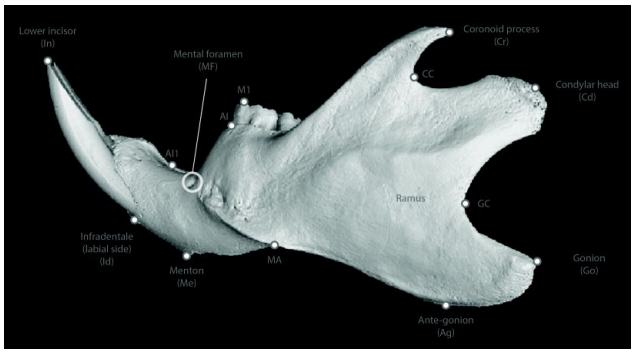

Fig. 1. Reference points for the estimation general size of the rat mandible CC: The upper mandibular noth, GC; lower mandibular notch, MA; the deepest point of the outer margin of bone that connects ME and Ag, AL; the higest point of the mesial alveolar bone at the lower first molar; AL; the deepest point of the outer margin of the bone M1; the highest point of the mesiobuccal cusp of the lower molar (adapted from Maki *et al*., 2012).

spaces (TbSp), and bone volume to total volume (BV/TV). The region of interest (ROI) looked at was between the 1st and 2nd molar teeth in the body of the mandible.

Tensile strength of the mandible. A Shimadzu universal testing machine was used for 3-point bending tests. The specimens (left hemi-mandibles) were placed on two rounded bars set 15 mm apart and a load was applied. These biomechanical tests assessed the following parameters: bending strength which is the amount of force required for the bone to start to bend; deflection which is the amount of vertical deviation from the horizontal plane required to facture the mandible; ultimate load which is the amount of force required to fracture the mandible; and time which refers to the duration that the mandible withstands the force until fracture.

Data Analysis. Data obtained was managed in Microsoft Excel 2021 (Microsoft Corporation). The data was analyzed using the Statistical Package for Social Service (SPSS) version 27 (IBM ®), 2020 software. The reliability of the data was observed by using Lin's concordance correlations for reliability. The test of normality for the data was done using the Shapiro-Wilk test. The t-test was performed as there were two groups that needed to be compared to each other. A p-value ≤ 0.05 was considered statistically significant, at a confidence interval of 95 %.

RESULTS

Blood alcohol concentration (BAC). The mean blood alcohol concentration (BAC) was 27.5 mg/dl in the 1-week alcohol exposed rats and 19.58 mg/dl in the 1-week pair-fed control rats. The mean BAC was 44.92 mg/dl in the 4-week alcohol exposed rats and 32.12 mg/dl in the 4-week pair-fed control rats.

Osteometric measurements. Differences in the general mandibular size were minimal (Figs. 2a and 2b) between the 1-week alcohol-exposed rats and the 1-weekpair-fed control rats in the acute binge model.

The distance between the mental foramen (MF) and the deepest point of the outer margin of bone (MF-MA) and the height of coronoid which is the distance between the coronoid (Cr) and ante-gonion (Ag) (Cr-Ag) were slightly higher in the 1-week pair-fed control rats (7.87 mm ± 0.05), (14.32 mm ± 0.21), when compared to the 1-week alcohol-exposed rats (7.33 mm ± 0.38), (14.28 mm ± 0.07). Cd-Id was slightly lower in in the 1-week pair-fed control rats (26.43 mm± 0.85) when compared to the 1-week-alcohol exposed rats (26.60 mm ± 0.09). No significant differences were seen in the above measurements (p=0.186 and p=0.853). Significant differences were however seen in the height of the condylar head (Cd-Ag) which was slightly higher in the 1-week-alcohol-exposed rats (12.83 mm ± 0.09) than in the 1-week pair-fed-control rats (12.25 mm ± 0.07) (p=0.02). We also observed a slightly greater height of the central portion of alveolar bone (AlI-Me) in the 1-week pair-fed controls (4.58 mm ± 0.02) than in the 1-week alcohol-exposed rats (4.35 mm ± 0.07) (p=0.047). Minor differences were seen between the 4-week alcohol-exposed rats and

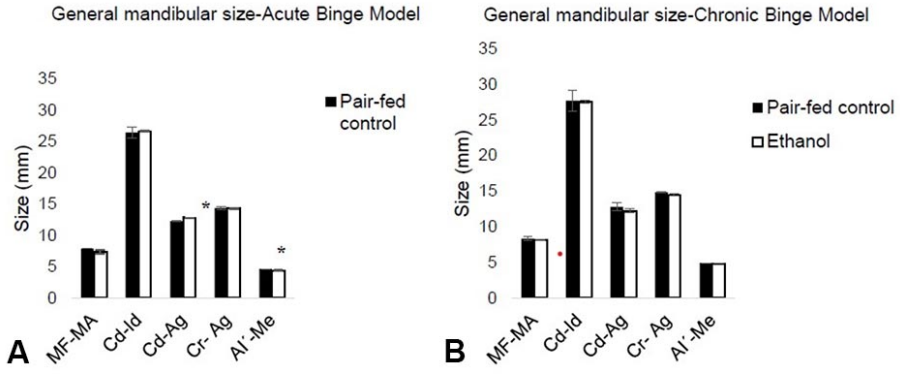

Fig. 2. Size estimation: (a) General mandibular size in the acute binge model; (b) General mandibular size in the chronic binge model MF-MA: Mental foramen-the deepsest point of the outer margin of bone. Cd-Id: Condylar head-infrandentale. Cd-Ag: Condylar head-Ante-gonion. Cr-Ag: Coronoid process-Ante-gonion. Al`-Me: Deepest point of the outer margin of the bone-menton. Mean Values are given and error bars represent standard deviation. *p<0.05.

the 4-week pair-fed control rats in the chronic binge model where MF-MA (8.33 mm ± 0.28), Cd-Id (27.68 mm ± 1.44), Cd-Ag (12.78 mm ± 0.59), Cr-Ag (14.82 mm ± 0.12), and AlI-Me (4.88 mm ± 0.02) were all slightly higher in C4 when compared to A4 (8.15 mm ± 0.02), (27.57 mm ± 0.19), (12.23 mm± 0.28), (14.47 mm ±0.14), and (4.83 mm ±0.00) respectively. No significant differences were seen in the measurements in the chronic binge model (p=0.457, p=0.625, p=0.356, p=0.115 and p=0.095) respectively.

Trabecular morphometry

Bone to total volume ratio (BV/TV). With regards to the bone to total volume ratio (BV/TV) in the acute and chronic binge models their patterns differed slightly. The BV/TV showed a slightly higher value in the 1-week pair-fed control rats (mean = 54 % ± 0.01) when compared to the 1-week alcohol-exposed rats (mean = 53 % ± 0.06) in the acute binge model, however, no significant differences were detected (p=0.97) (Fig. 3a). The pattern exhibited in the chronic binge group was different, where the 4-week alcohol-exposed rats displayed a higher BV/TV (mean = 59 % ±0.01) in comparison to the 4-week pair-fed control rats (mean = 55 % ± 0.06), however no significant differences were observed (p=0.43).

Trabecular thickness (TbTh). In the acute binge model, the trabecular thickness (TbTh) was similar between the 1-week pair fed control (mean = 0.12 mm ±0.02) and 1-week alcohol exposed rats (mean = 0.10 mm ±0.01) (p=0.60). The same pattern was seen in the chronic binge model where the 4-week pair-fed control rats (mean = 0.128 mm ±0.002) had a similar trabeculae thickness to the 4-week-alcohol exposed rats (mean = 0.132 mm ±0.01) (p=0.77) (Fig. 3b).

Trabecular spacing (TbSp). In the acute binge model, the trabecular spacing (TbSp) was similar in the 1-week pair-fed control rats (mean = 0.10 mm ±0.01) and the 1-week alcohol-exposed rats (mean = 0.09 mm ±0.01) (p=0.58). A similar pattern was observed in the chronic binge model, where the trabecular spaces were similar in the 4-week pair-fed control rats (mean = 0.10 mm ±0.01) and the 4-week alcohol-exposed rats (mean= 0.09 mm ±0.004) (p=0.28) (Fig. 3c).

Trabecular number (TbN). The mean trabecular number (TbN) was slightly higher in the 1-week alcohol-exposed rats (mean = 5.09 mm-1 ±0.05) when compared to the 1-week pair-fed control rats (mean = 4.69 mm-1 ±0.78) in the acute binge model, however no significant difference was seen (p=0.56). A similar pattern was observed in the chronic binge group with more trabeculae seen in the 4-week alcohol-exposed rats (mean = 4.48 mm-1 ±0.13) than in the 4-week pair-fed control rats (mean = 4.24 mm-1 ±0.02) (p=0.12) (Fig. 3d).

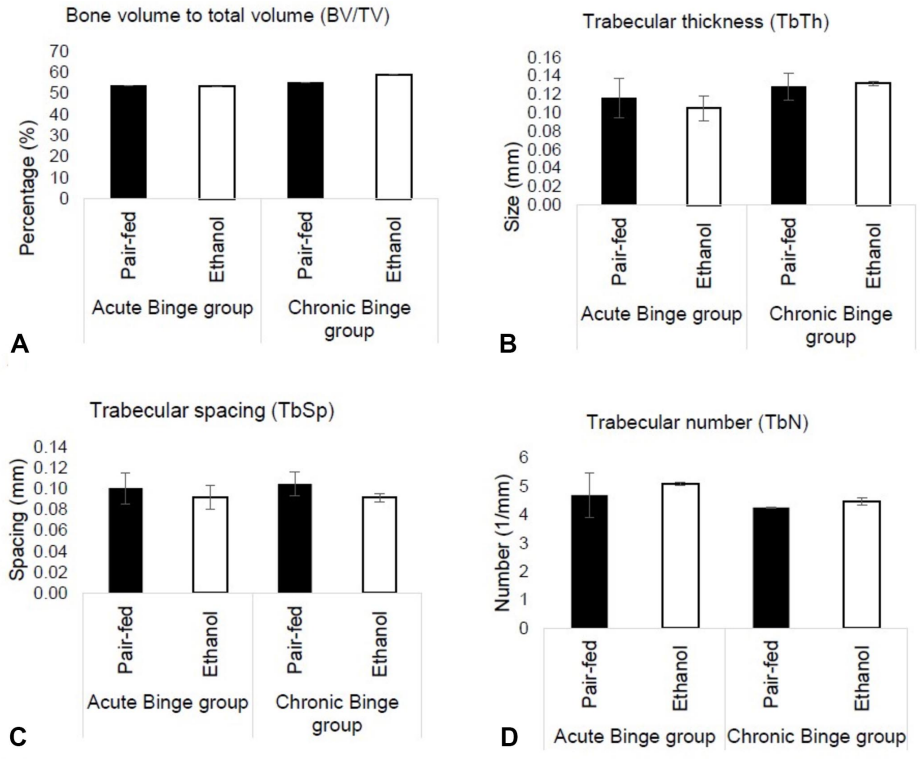

Fig. 3. Trabecular morphometric parameters, values are represented as means; (A), bone to total volumen (BV/TV); (B), Trabecular thickness (TbTh); (C) Trabecularspacing (TbSp); (D), trabecular number (TbN). Error bars represent standard deviation.

Tensile strength of the mandible

Acute binge group. The maximum force and break force were greater in the 1-week alcohol-exposed rats than in the 1-week pair-fed control rats. With respect to the amount of time required to fracture the mandibles when applying force (maximum time), the 1-week pair-fed control rats took longer to fracture. The maximum displacement was similar in both groups. The displacement force was less in the 1-week pair-fed control rats than it was in the 1-week alcohol-exposed rats. (Table II).

Chronic binge group. The maximum force and break force were greater in the 4-week alcohol-exposed rats than in the 4-week pair-fed control rats. The maximum time was slightly greater in the 4-week alcohol-exposed rats. The maximum displacement was similar in both groups. The displacement force was slightly less in the 4-week pair-fed control rats than it was in the 4-week alcohol-exposed rats (Table II).

Table II. Tensile strength of the mandible.

	Acute Binge Group				Chronic Binge Group			
Parameter	Group	Mean	SD	P-value	Group	Mean	SD	P-value
Maximum force (N)	Pair-fed	66.27	3.62	p=0.1	Pair-fed	92.84	10.53	p=0.27
	Ethanol	80.66	6.02		Ethanol	106.53	7.08	
Maximum displacement (mm)	Pair-fed	2.68	0.11	p=0.055	Pair-fed	1.93	0.05	p=0.49
	Ethanol	2.34	0.05		Ethanol	2.03	0.17	
Maximum time (s)	Pair-fed	53.58	2.16	p=0.055	Pair-fed	38.54	1.04	p=0.49
	Ethanol	46.80	0.91		Ethanol	40.66	3.44	
Break force (N)	Pair-fed	62.78	5.04	p=0.08	Pair-fed	92.84	10.53	0.26
	Ethanol	80.66	6.02		Ethanol	106.30	6.74	
Displacement force (N)	Pair-fed	1.69	1.58	p=0.097	Pair-fed	2.03	2.28	p=0.86
	Ethanol	5.11	0.39		Ethanol	2.43	1.52	

DISCUSSION

We sought to investigate whether a dose of 1.5 g/kg of 20 % (vol/vol) alcohol solution would be sufficient to mimic a binge drinking model in male adolescent Sprague Dawley rats. We also wanted to determine if this dose would influence the growth and development of the mandible in any way by examining the effect on bone strength, general bone size, and trabecular parameters. A dose of 1.5 g/kg of a 20 % (vol/vol) alcohol solution did not result in concentrations of alcohol in the blood that are high enough to mimic a binge drinking model.

Osteometric measurements showed significant height differences in the condylar head (Cd-Ag) where the 1-week alcohol-exposed rats had a greater height than the 1-week pair-fed control rats, whereas the central portion of the alveolar bone (Al´-Me) was slightly greater in the 1-week pair-fed control rats when compared to the 1-week alcohol-exposed rats (in the acute binge model). This shows conflicting results. The Al´-Me measurement being greater in the pair-fed control group correlates with numerous studies which have showed a decrease in osteometric parameters in alcohol-exposed rats in both chronic and binge drinking models, suggesting an interference in growth of the adolescent mandible (Sampson *et al*., 1996; Wezeman *et al*., 1999; Lauing *et al*., 2008; Rosa *et al*., 2019). The increase in size of the Cd-Ag measurement in the alcohol group contradicts the effects seen in previous studies (Sampson *et al*., 1996; Wezeman *et al*., 1999; Lauing *et al*., 2008; Rosa *et al*., 2019), where alcohol consumption causes a decrease in bone size. The increase in size may be due to the BAC in this study being lower than the amount needed for a binge alcohol model. Light to moderate alcohol consumption has been seen to have beneficial effects on various body systems (Le Daré *et al*., 2019). No significant differences were seen in the trabecular morphometry. This is likely due to the lower concentration of alcohol used in this study, as well as the small sample size. Föger-Samwald *et al*. (2018), reported on the effect of alcohol on trabecular morphometry in different bones of prepubescent pigs exposed to a binge drinking model. The results showed a decrease in TbN in the alcohol exposed groups in the femur and humerus (Föger-Samwald *et al*., 2018). A decrease in TbTh and increase in TbSp was seen in a study which investigated the effect of binge drinking on adolescent alveolar bone. Alcohol is known to have a negative effect on the trabecular morphometry by decreasing both osteoblast number and function which decreases cancellous bone volume and cortical bone strength which will result in an increased risk of fractures (Wezeman *et al*., 1999).

The strength of the bone was not affected in the current study. Again, this is likely attributed to the lower

concentration of alcohol used in this study, along with the small sample size. A study by Lauing *et al*. (2018) reported no decrease in compressive strength of the vertebra of rats after a week of binge alcohol exposure similar to the current study. However, a decrease was seen in compressive strength in rats exposed for 4 weeks of binge alcohol exposure (Lauing *et al*., 2008). A decrease in compressive strength can also lead to weaker bones that have a greater risk of fracture. This also shows that the effect of binge drinking may be dependent on both dosage and exposure time.

CONCLUSION

A dose of 1.5 g/kg of a 20 % (vol/vol) alcohol solution did not bring about concentrations needed to classify this as a binge drinking alcohol model. The dosage used in this study only inferred minor changes in bone size but had no impact on bone strength and trabecular parameters. Previous studies by Pillay & Ndou (2021), Zhao *et al*. (1998), and Kasdallah-Grissa *et al*. (2007), on the effect of alcohol on rats made use of a 25.2 % (vol/vol) alcohol solution at a dose of 3 g/kg, a 20 % alcohol solution at a dose of 3 g/kg and a 35 % (vol/vol) alcohol solution at a dose of 3 g/kg respectively (Zhao *et al*., 1998; Kasdallah-Grissa, *et al*., 2007; Pillay & Ndou, 2021). For this current study to elicit BAC's which fall in the range to mimic a binge drinking model, the dose should be increased from 1.5 g/kg to at least 3g/kg. The lack of significant changes seen between the alcohol and pair-fed control groups in both acute and chronic binge exposed rats is likely due to the low dose of alcohol administered to the rats in the study. The small sample size will only be considered as a secondary limitation to the desirable dose.

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RESUMEN: El consumo excesivo de alcohol en adolescentes tiene un efecto negativo en el desarrollo del esqueleto y en la consecución de la masa ósea máxima. Nuestro estudio tuvo como objetivo examinar el efecto del consumo excesivo de alcohol sobre el crecimiento y la integridad funcional de la mandíbula de la rata adolescente Sprague Dawley y determinar si una dosis de 1,5 g/kg es suficiente para producir un modelo de consumo compulsivo. Un total de ocho ratas Sprague Dawley adolescentes (machos) de 7 semanas de edad se colocaron aleatoriamente en 4 grupos con dos ratas cada uno: ratas expuestas al alcohol durante 1 semana, ratas de control alimentadas en parejas durante 1 semana, ratas expuestas al alcohol durante 4 semanas, y ratas de control alimentadas en parejas durante 4 semanas. A los grupos expuestos al alcohol se les administró una dosis única diaria mediante sonda oral de 1,5 g/kg de alcohol al 20 % 3 días a la semana (días alternos) durante 7 o 28 días. A los grupos de control alimentados por parejas se les administró una dosis calórica equivalente de maltosa dextrina mediante sonda oral los mismos días que a las ratas expuestas al alcohol. Las ratas expuestas al alcohol durante una semana, las ratas de control al día 7, las ratas expuestas al alcohol durante cuatro semanas y las ratas de control al día 28. Se diseccionaron las mandíbulas y se determinaron las mediciones osteométricas utilizando un calibre vernier digital. Los huesos se escanearon utilizando un escáner 3D-microCT (Nikon XTH 255L). Las pruebas biomecánicas se realizaron utilizando una máquina de pruebas universal Shimadzu. Las diferencias observadas se relacionaron con la osteometría mandibular, que mostró una altura reducida en la porción central del hueso alveolar (Al'-Me) y un aumento en la altura de la cabeza condilar (Cd-Ag) en las ratas expuestas al alcohol durante una semana, en comparación con las ratas control alimentadas en parejas durante una semana. No se observaron otras diferencias. La falta de diferencias significativas entre los grupos de alcohol y de control alimentados en parejas expuestas a ebriedad aguda y ebriedad crónica, probablemente se deba a la baja dosis de alcohol administrada a las ratas en el estudio, por lo que se propone una dosis más alta.

PALABRAS CLAVE: Ebriedad de alcohol; Crecimiento esquelético; Osteometría; Prueba biomecánica; Mandíbula adolescente.

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Corresponding author:
Akaashni Bhika
School of Anatomical Sciences
Faculty of Health Sciences
University of the Witwatersrand
7 York Road
Parktown
Johannesburg 2193
SOUTH AFRICA

E-mail: akaashni.bhika@wits.ac.za