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Mandibular Trabecular Morphometry and Tensile Strength Assessment in the Diabetic Sprague Dawley Rat Consuming Alcohol

Morfometría Trabecular Mandibular y Evaluación de la Resistencia a la Tracción en Ratas Diabéticas Sprague Dawley que Consumen Alcohol

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SUMMARY: Both alcohol abuse and diabetes are associated with osteoporosis and high fracture rate. However, studies on the combined effects of alcohol and diabetes on the mandible are lacking in the scientific literature. Therefore, this study aimed to investigate the impact of concurrent alcohol intake on the trabeculae morphometry and tensile strength of diabetic rat mandibles. Twenty-nine male adult Sprague-Dawley rats were respectively grouped as: untreated (control) (n=8); alcohol (ALC) (n=8); diabetic (DBT) (n=7); diabetic animals treated with alcohol (DBT+ALC) (n=6). Diabetes was induced through a high fructose diet and streptozotocin and confirmed through serum insulin levels and blood fasting glucose. The rats that were receiving alcohol were given 10 % v/v alcohol daily in drinking water for 84 days. After 12 weeks, bilateral hemi-mandibles were harvested and fixed in 10 % buffered formalin before conducting microtomography to assess trabecular morphometric parameters and tensile strength. The results revealed that trabecular parameters and tensile strength were negatively impacted. The ALC group had more BV/TV, which contradicts previous studies suggesting that alcohol can alter trabecular morphological parameters. The DBT group had lower maximum and breaking forces, suggesting that alcohol had no adverse effect on maximum and breaking force when used independently. The highest stiffness was observed in the DBT+ALC rats. However, the bone quality was lower, indicating that alcohol consumption may not be a significant factor in bone health.

KEY WORDS: Osteoporosis; Bone fracture; Alcoholism; Diabetes mellitus; Mandible.

INTRODUCTION

The burden of disease can be worsened by non-communicable diseases, which is exacerbated by alcohol and substance abuse in low and middle-income countries (Samodien *et al.*, 2021). The abuse of alcohol is relatively high in parts of Africa with the prevalence of alcohol use disorder ranging from 1-32 % when considering sub-Saharan Africa (Gellé *et al*., 2023). Diabetes mellitus is increasingly becoming more prevalent and is a significant health challenge to society and authorities in various jurisdictions (Motala *et al*., 2022). This requires more resources to be directed at addressing non-communicable diseases as well as alcohol and other substances abuse.

Individually, diabetes (Romero-Díaz *et al*., 2021) and alcohol abuse (Godos *et al*., 2022) are associated with skeletal pathology. Previous research has linked diabetes to osteoporosis and increased risk of bone fractures (Lin *et al*., 2021). In rodents, diabetes reduces osteometric and trabecular morphometric parameters such as thickness and number of trabeculae in bone (Reinwald *et al*., 2009; Ndou *et al*., 2024). Diabetes can also weaken bones by affecting the body's ability to produce new bone tissue and the repair of damaged bones (Tomasiuk *et al*., 2023). It is well established in the scientific literature that alcohol consumption has detrimental effects on bone health (Cho *et al*., 2018; Ndou *et al*., 2023). Abuse of alcohol can lead to decreased bone density, increased risk of fractures, and impaired bone healing (Godos *et al*., 2022).

Alcohol contributes to the causes of osteoporotic fractures which rise with increased alcohol consumption (Godos *et al*., 2022). Similarly, diabetes mellitus is associated with osteoporosis (Paschou *et al*., 2017). In the mandible, osteoporosis can lead to periodontal bone loss

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and eventual loss of teeth, which may require dental implants as a treatment option (Esfahanian *et al*., 2012). The viability of dental implants is dependent on the amount of cortical and trabecular bone present, and the formation of new bone (Porter & von Fraunhofer, 2005). The implant tends to fail due to a lack of osseointegration in individuals suffering from conditions that enhance bone degeneration, such as osteoporosis (Beikler & Flemmig 2003; Stevenson *et al*., 2007; Hasegawa *et al*., 2008; Yarramsetty *et al*., 2023).

It is plausible to postulate that alcohol abuse among diabetics would worsen the detrimental effects on skeletal health. Alcohol may exacerbate the bone-weakening effects of diabetes by further disrupting bone turnover and mineralization. Additionally, alcohol may worsen the complications of diabetes, leading to poor circulation and nerve damage, which may impair bone healing and increase the risks of fractures and osteoporosis.

Alcohol abuse among individuals with diabetes may have a serious impact on bone health, particularly on the mandible trabeculae and tensile strength. Understanding how these factors impact bone density and structure is crucial for assessing the risks associated with alcohol abuse or diabetes, individually, and the case of alcohol abuse and diabetes comorbidity. This study sought to establish whether the interaction of alcohol and diabetes would exacerbate the risk of bone-related complications regarding mandibular trabeculae, and bone strength.

Therefore, the current study investigated the combined effect of type 2 diabetes and excessive alcohol intake using Sprague-Dawley rats as a model. The study used Microfocus X-ray Computed Tomography (MicroCT) to determine possible microstructural alterations. Therefore, mandibular trabeculae morphometry such as bone volume fraction (BV/TV), trabecular thickness (TbTh), spacing (TbSP), and number (TbN) were evaluated. Additionally, the tensile strength was tested using a universal tensile tester.

MATERIAL AND METHOD

Ethics. Ethics approval was obtained from the Animal Ethics Committee of the University of the Witwatersrand, Johannesburg (AESC: 2018/011/58/C) and the procedures were aligned with the standards laid out by this committee.

Animal housing. The study comprised 29 male adult Sprague-Dawley rats weighing approximately 330g-370g. The rats were kept in the Central Animal Services (CAS), University of the Witwatersrand, Johannesburg, South Africa. These animals were kept in pathogen-free conditions and housed in plastic cages individually, with unlimited rodent diet and water. They were kept under standard animal house conditions (temperature: 21-23?C, light approximately 12 hours light-dark cycle).

Treatment of animals and group allocation. The animals were placed in the following groups: untreated (control) which received no treatment (n=8); alcohol group (ALC) (n=8), given 10 % v/v alcohol daily in drinking water for 84 days; diabetic group (DBT) (n=7); and diabetic animals treated with alcohol (DBT+ALC) (n=6).

Diabetes induction. Rats were fed rodent diet modified with 20 % fructose (Nature's Choice™, Whole Food Specialists, Randvaal, South Africa) for two weeks before receiving a single injection of 40 mg/kg streptozotocin (STZ) (Sigma, St. Louis, MO, USA) in freshly prepared citrate buffer at 0.05 M (pH 4.5).

Termination procedures. At the end of 12 weeks, the animals were deeply anesthetized with pentobarbitone. Blood samples were collected by cardiac puncture in serum-separating tubes (4ml) then centrifuged at 3,000 rpm for 10 minutes. Serum was decanted into aliquots that were stored at –80°C. The mandible was meticulously dissected, bisected into left and right hemimandibles, and fixed in 10 % buffered formalin for further processing.

Fasting glucose tests and terminal insulin analysis. The animals were fasted overnight before taking readings using a glucometer (Accucheck®, Roche Diagnostics, Germany) once a week to monitor diabetes onset and progression of diabetes. Glucose values greater than 250 mg/dL were considered diabetic. Serum analysis of insulin was conducted by ELISA (Insulin ELISA kit; Elabscience).

Three-Dimensional Microfocus X-ray Computed Tomography (MicroCT). A Nikon XTH 225/320 LC X-ray microtomography was used to scan the mandibles for computed tomography. The bones were secured in oasis floral foam to keep the samples steady during scanning and positioned on the 360? rotating sample manipulator. The X-ray settings were standardized to a source voltage of 70kV and a source current of 400µA and a four-frame average was used to improve the signal-to-noise ratio. Both hemimandibles from each rat were scanned at a resolution of 18µm. The volume Graphics® 3.2 software was then used for the analysis of the following parameters: bone volume fraction (BV/TV), trabecular thickness (TbTh), spacing (TbSP), and number (TbN). ROI was defined as the distance from the start point set on the mesial surface of the first molar (M1) and the endpoint set at the distal surface of the third molar (M3) (Fig. 1).

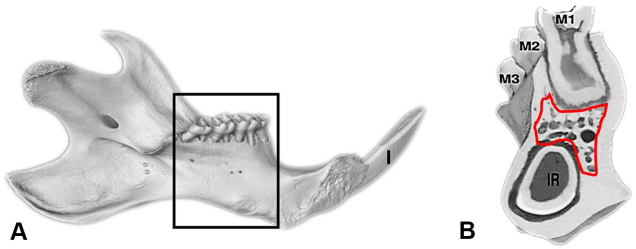

Fig. 1. Region of interest (ROI). A three-dimensional rendering of the rat hemimandible. The boxed area shows a mediolateral view of the ROI. B: Cross-sectional anteroposterior view of the mandible showing the ROI (bounded area). I, incisor tooth; IR, incisor root; M1 M2 M3, molar teeth.

Tensile strength test (3-point bending). A Shimadzu universal test machine was utilized for mandibular tensile strength (EZ-X S 200V E SSM346-57320-44, Shimadzu, Honeydew, South Africa). To do this, the left hemi-mandibles with the lingual side facing up, were placed on two rounded bars set at 15 mm apart and the load was applied. The load-displacement curves were then recorded at 3mm/min until failure.

Data analysis. Data were managed in Microsoft Excel, and Office 365 (Microsoft Corporation®) and analyzed using SPSS® version 28 (IBM®). ANOVA with Tuckey post hoc was used for multiple group comparisons of means for the tensile strength and trabecular morphometric parameters. The results were represented as means and standard deviation. The significance level was established at a confidence interval of $p < 0.05$.

RESULTS

Fasting blood glucose. Fasting blood glucose level in non-diabetic experimental groups was similar to untreated controls, as the ALC (74.10mg/dL±14.82) did not show statistical significance compared to the untreated (79.80 mg/dL±6.63) ($p > 0.999$) (Fig. 2A). On the contrary, fasting blood glucose levels were significantly higher in DBT (312.3mg/dL±152.9), DBT+ALC (251.4mg/dL±124,6) ($p=0.002$ and $p=0.046$, compared to untreated, respectively) (Fig. 2B).

Serum insulin concentration. Serum insulin levels detected in the ALC (1152 pg/mL±150.3) were similar to the untreated control group (1251 pg/mL±47.2) ($p=0.760$) (Fig. 2A). On the contrary, the DBT (771.2 pg/mL±146.2) and DBT+ALC (693 pg/mL±52.87) had significantly lower insulin levels than the untreated control group ($p<0.001$ for both comparisons) (Fig. 2B).

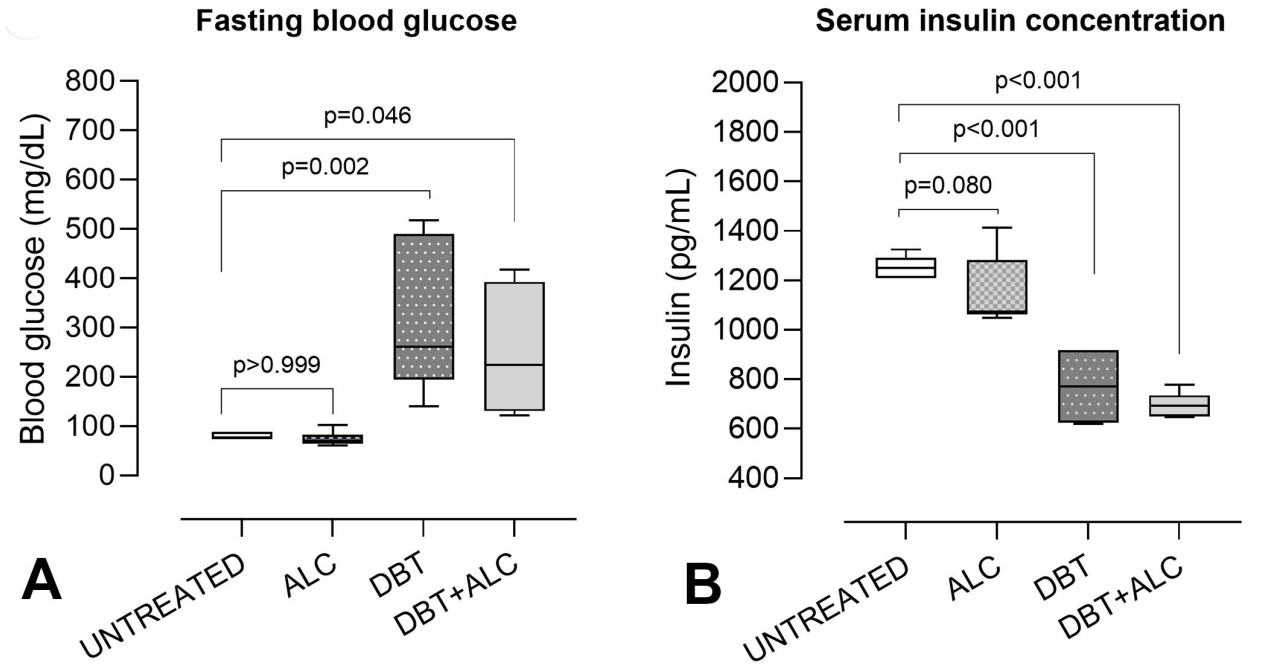

Fig. 2. Blood test results at the end of 12 weeks for all the groups in the study. A, fasting blood glucose. Both the diabetic groups had higher fasting blood glucose than the untreated control. The fasting blood glucose for ALC was similar to the untreated control. B, Serum insulin concentration. Both the diabetic groups had lower insulin concentration than the untreated control. The ALC, group had similar insulin concentration to the untreated control. Box-and-whisker plots of adipocyte quantity in various study groups. The line inside the box represents the mean, whereas the bottom and top lines of the box show the 25th and 75th percentiles, respectively. The whiskers below and above the box show the minimum and maximum values, respectively. Untreated control; ALC, alcohol; DBT, diabetic; DBT+ALC, diabetes and alcohol

Trabecular bone morphometric parameters

Bone volume to fraction (BV/TV): The ALC group had the highest bone-to-total volume ratio (BV/TV) which was significant compared to the untreated control group (p≤0.001). Conversely, the BV/TV in the DBT and DBT+ALC was similar to the untreated group (p=0.474 and p=0.548, respectively) (Table I). Regarding the alcohol effects, the ALC group had a significantly higher BV/TV than the DBT, and DBT+ALC groups (p=0.017 and p ≤ 0.001, respectively (Table I). However, theDBT+ALC showed similarities with the DBT group (p=0.934) (Table I).

Trabecular thickness (TbTh): None of the study groups displayed significant differences in comparison to the untreated control (p>0.05) (Table I). Also, the ALC group had a similar trabecular thickness to the DBT+ALC group except when compared to the DBT group which showed more trabeculae thickness than the ALC group (p=0.022). The DBT group had a similar trabecular thickness to the DBT+ALC group (p=1.07)

Trabecular number (TbN): No treatment groups showed significant differences in TbN when compared to the untreated control (p>0.05) (Table I). The ALC group exhibited similarities in TbN when compared to all groups (p>0.05). Also, the DBT+ALC group showed similarities with the DBT group (p>0.05) (Table I).

Trabecular spacing (TbSp): None of the study groups showed any significant differences in the trabecular spacing (TbSp) when compared to the untreated control (p>0.05) (Table I). Furthermore, all other group comparisons showed similarities except for the smaller trabecular spacing (TbSp) exhibited in the DBT group compared to the DBT+ALC group (p=0.035).

Tensile strength

Mean maximum force: The DBT group showed the lowest values for maximum force which were statistically significant compared to the untreated control (p<0.001). No other groups exhibited significant differences from the untreated group (p?0.05) (Table II). The ALC group had a significantly higher value than the DBT group (p≤0.001). The DBT group showed a significantly lower value than the DBT+ALC group (p<0.001) (Table II).

Mean break force: The DBT group exhibited significantly lower break force than the untreated group (p<0.001) (Table II). On the contrary, the ALC and DBT+ALC treated groups did not show statistical differences from the untreated group (p=0.119 and p=0.256, respectively) (Table II). The ALC group had a significantly higher break force than the DBT group (p≤0.001). The DBT group required substantially less force to fracture than the DBT+ALC (p<0.001).

Mean displacement. Lower displacement values were recorded among the DBT+ALC and DBT-treated groups compared to the untreated control (p=0.001, p=0.007, respectively) (Table II). No other groups showed significant differences in comparison to the untreated control (p?0.05).

Meantime. The DBT+ALC and DBT-treated groups required the shortest time to fracture the mandible which was significant compared with the untreated control (p=0.003 and p=0.006, respectively). No other groups showed significant differences in comparison to the untreated control (p?0.05) (Table II).

Table I. Trabecular morphometric parameters.

	Untreated		ALC		DBT		DBT+ALC	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
N	16		16		14		12	
[a]BV/TV	58.13	4.96	67.50	4,89	56.65	4,61	56.83	7,28
*p-value			p≤0.001		0,474		0,548	
[b]TbTh	0,45	0,09	0,39	0,06	0,67	0,89	0,47	0,17
*p-value			0.615		0.068		0,925	
[c]TbN	2,00	0,26	2.00	0.26	2,02	0,27	2,09	0,49
*p-value			0.503		0,691		0,873	
TbSp	0.06	0.02	0,10	0,02	0,05	0,02	0,11	0,25
*p-value			0,142		0,602		0,091	

[a]BV/TV: ALC vs DBT+ALC (p<0.001); DBT vs ALC, DBT+ALC (p<0.001 and p=0.934, respectively)
[b]TbTh: ALC vs DBT+ALC (p=0.575); DBT vs ALC, DBT+ALC (p=0.022 and p=0.107, respectively)
[c]TbN: ALC vs DBT+ALC (p=0.873); DBT vs ALC, DBT+ALC (p=0.802 and p=0.698, respectively)
[d]TbSp: ALC vs DBT+ALC (p=0.740); DBT vs ALC, DBT+ALC (p=0.053 and p=0.035, respectively)
Comparison of respective groups with the untreated group. ALC, alcohol; DBT, diabetic; DBT+ALC, diabetes and alcohol.

Mean stiffness. The mean stiffness among the treatment groups showed a varied pattern compared to the untreated control, with the DBT group exhibiting less stiffness than the untreated control (p=0.006). No other groups showed significant differences compared to the untreated control (p?0.05) (Table II).

Regarding alcohol administration, the ALC group had significantly higher stiffness than the DBT group (p= 0.008). Concerning diabetes, the DBT group had significantly less stiffness than the DBT+ALC group (p<0.001) (Table II).

Table II. Tensile strength parameters from 3-point bending tests.

	Untreated		ALC		DBT		DBT+ALC	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
N	16		16		14		12	
[a]Max Force(N)	117,65	34,24	104,85	31,31	71,06	25,14	106,30	22,65
*p-value			0,191		<0,001		0,273	
[b]Break Force (N)	117,65	34,24	102,60	29,16	72,66	27,43	105,83	22,65
p-value			0.119		<0,001		0.256	
[c]Displacement (mm)	2,08	0,43	1,84	0,22	1,69	0,29	1,58	0,42
*p-value			0,083		0,007		0,001	
[d]Time (sec)	40,81	7,43	36,70	4,45	33,02	6,40	31,52	8,45
p-value			0.143		0.006		0.003	
[e]Stiffness (N/mm)	59,61	19,08	58,79	21,20	43,86	15,61	70,36	18,77
*p-value			0,905		0,006		0,148	

[a]Max force: ALC vs DBT+ALC (p=0.890); DBT vs ALC, DBT+ALC (p<0,001 and p<0,001, respectively).
[b]Break force (N): ALC vs DBT+ALC (p=0.755); DBT vs ^ALC, DBT+ALC (p<0,001, for both comparisons).
[c]Displacement: ALC vs DBT+ALC (p=0.092); DBT vs ALC, DBT+ALC (p=0.286 and p=0.515, respectively).
[d]Time: ALC vs DBT+ALC(p=0.092); DBT vs ALC, DBT+ALC (p=0.168 and p=0.515, respectively).
[e]Stiffness: ALC vs DBT+ALC (p=0.119); DBT vs ALC, DBT+ALC (p=0.008 and p<0,001, respectively).
* Comparison of respective groups with the untreated group; ALC, alcohol; DBT, diabetic; DBT+ALC, diabetes and alcohol

DISCUSSION

The main aim was to test the hypothesis that the combination of diabetes and alcohol may have a more negative impact on the mandible than the singular effects of alcohol, or diabetes by causing adverse effects on trabecular morphology, and tensile strength of the mandible. The internal architecture was assessed from the data obtained using microfocus X-ray computed tomography. We found altered trabecular parameters and the tensile strength to be negatively impacted using 3-point bending tests in diabetic rats and when alcohol was given to diabetic rats.

Aspects of bone trabecular morphometric parameters

In the present study, the ALC group had much more BV/TV than the other animal groups. This is contrary to previous studies which have demonstrated that trabecular morphological parameters have been altered by alcohol consumption (Frazão *et al*., 2020). It is possible that the amount of alcohol used in this investigation was too low to cause any harmful effect on trabecular morphometry. In a study by Godos *et al*. (2022), it was suggested that low to moderate alcohol consumption leads to increased bone mineral density while heavy alcohol consumption affects bone mineral density (Godos *et al*., 2022; LeBoff *et al*., 2022). Furthermore, this study allowed unlimited access to rodent diets that could have provided adequate nutrition. Alcohol addicts are known for poor diets (Mutuli *et al*., 2020). The low alcohol consumption and balanced diet given to the rat models in this study could have mitigated any side effects associated with alcohol consumption.

When diabetic rats received alcohol (DBT+ALC), there was more trabecular spacing (TbSp) compared to when the rats were diabetic, without other additional treatment (DBT). This suggests that alcohol may have disrupted the bone internal morphology when given to diabetic rats (DBT+ALC). Since it is established that diabetes or alcohol independently affect bone, this increase in trabecular spacing means that there was a confounding effect of alcohol when given to diabetic rats (Romero-Díaz *et al*., 2021; Godos *et al*., 2022).

Diabetic rats (DBT) and diabetic rats that received alcohol (DBT+ALC) had significantly lower bone fraction (BV/TV) than the group that received alcohol only (ALC). This supports previous literature reporting on the

detrimental effects of diabetes or alcohol on bone (Lin *et al*., 2021). A lower BV/TV is more likely to weaken bone, as shown by studies that report a higher incidence of fractures in diabetics (Ndou *et al*., 2024). We have not found comparable scientific literature of the bone effects of alcohol among diabetics.

Bone tensile strength. The maximum and breaking forces were lower in the DBT group than in the untreated control group. These parameters reflect how much load a bone can take before weakening and how much load or force fractures the mandible once weakened. These values were lowest in the DBT group. This was unexpected, as we hypothesized that ALC could confound the diabetic effects on bone.

The lack of significant differences for the ALC group compared to the untreated controls suggests that alcohol had no adverse effect on maximum and breaking force when used independently. Many studies report that alcohol is detrimental to bone health, as it weakens bone through osteoporosis in a dose-dependent manner (Godos *et al*., 2022; Ke *et al*., 2023). Therefore, the alcohol dosage in the present study may have been below the threshold for statistically detectable bone deterioration.

The stiffness was highest in the diabetic animals treated with (DBT+ALC) suggesting the better ability to resist deflection under load as corroborated by the less displacement recorded for these groups. However, this group showed less time to fracture. This is characteristic of bones that are both stiff and brittle, a feature observed in osteoporotic bones (Turner, 2006). Individually, diabetes (Romero-Díaz *et al*., 2021) and alcohol abuse (Godos *et al*., 2022) are associated with skeletal pathology.

Stiff bones are characterized by less ability to bend and less time to fracture. In the present study, diabetic rats that received alcohol (DBT+ALC) had less time to fracture accompanied by low displacement values than control rats. This demonstrates that diabetes with alcohol abuse could contribute to bone brittleness.

CONCLUSIONS

Diabetes and alcohol ((DBT+ALC) rats showed poor values for most trabecular morphometry and tensile strength tests than rats in the alcohol only (ALC) or diabetic group (DBT). This study showed that the interaction of alcohol and diabetes exacerbates the risk of bone-related complications regarding mandibular trabeculae, and bone strength. It is advisable for clinicians to recommend reduced alcohol used among diabetic patients.

EGUAVOEN, I.; MBAJIORGU, E.F.; PERRY, V.; PILLAY, D. y NDOU, R. Morfometría trabecular mandibular y evaluación de la resistencia a la tracción en ratas diabéticas Sprague Dawley que consumen alcohol. *Int. J. Morphol., 42(6)*:1706-1712, 2024.

RESUMEN: Tanto el abuso de alcohol como la diabetes están asociados con la osteoporosis y una alta tasa de fracturas. Sin embargo, faltan estudios en la literatura científica sobre los efectos combinados del alcohol y la diabetes en la mandíbula. Por lo tanto, este estudio tuvo como objetivo investigar el impacto de la ingesta simultánea de alcohol en la morfometría de las trabéculas y la resistencia a la tracción en las mandíbulas de ratas diabéticas. Veintinueve ratas Sprague-Dawley adultas macho se agruparon respectivamente: sin tratamiento (control) (n = 8); alcohol (ALC) (n = 8); diabética (DBT) (n = 7); Animales diabéticos tratados con alcohol (DBT+ALC) (n=6). La diabetes se indujo mediante una dieta alta en fructosa y estreptozotocina y se confirmó mediante los niveles de insulina sérica y glucosa en ayunas. Las ratas recibieron alcohol al 10 % v/v diariamente en el agua potable durante 84 días. Después de 12 semanas, se extrajeron las hemimandíbulas bilaterales y se fijaron en formalina tamponada al 10 % antes de realizar una microtomografía para evaluar los parámetros morfométricos trabeculares y la resistencia a la tracción. Los resultados revelaron que los parámetros trabeculares y la resistencia a la tracción se vieron afectados negativamente. El grupo ALC tuvo más BV/TV, lo que contradice estudios previos que sugieren que el alcohol puede alterar los parámetros morfológicos trabeculares. El grupo DBT tuvo fuerzas máximas y de rotura más bajas, lo que sugiere que el alcohol no tuvo un efecto adverso sobre la fuerza máxima y de rotura cuando se usó de forma independiente. La mayor rigidez se observó en las ratas DBT+ALC. Sin embargo, la calidad ósea fue menor, lo que indica que el consumo de alcohol puede no ser un factor significativo en la salud ósea.

PALABRAS CLAVE: Osteoporosis; Fractura ósea; Alcoholismo; Diabetes mellitus; Mandíbula.

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