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ORIGINAL ARTICLE

Catha edulis and Cathinone Effect on Bone Parameters and Adipokines Gene Expression in Obese Male Mice

Mustafa Ahmed Alshagga[1,2] · Zahurin Mohamed[1] · Norazlina Mohamed[3] · Atefehalsadat Seyedan[1] · Yan Pan[2] · Sharoen Yu Ming Lim[2] · Mohammed Abdullah Alshawsh[1,4]

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Abstract

This study aims to determine the effects of *Catha edulis* (Vahl) Forssk. ex Endl., Celastraceae, and its bioactive alkaloid, cathinone, on liver histology, gene expression of inflammatory biomarkers in white adipose tissue, hypothalamus, and histomorphological bone parameters. Thirty male mice (C57BL/6J) were fed a high-fat diet for 8 weeks, divided into 5 groups, and treated daily for another 8 weeks high-fat diet + vehicle (control), HFD + 15 mg/kg orlistat, high-fat diet + 200 mg/kg plant extract, high-fat diet + 400 mg/kg plant extract, and high-fat diet + 3.2mg/kg cathinone. Treatments were carried out once daily by gastric gavage. Blood and tissue samples were collected for histological, biochemical, and gene expression analysis. The results showed the plant extract (400 mg/kg) and cathinone (3.2 mg/kg) displayed a marked reduction of liver fatty changes. All treated groups showed higher serum adiponectin levels compared to the control group (p <0.05). Plant extract–treated groups showed upregulation of adiponectin gene expression in white adipose tissue, however, at the concentration of 200 mg/kg upregulated interleukin-6 in the hypothalamus; in addition, this group showed less-eroded surfaces and higher bone mass compared to control. Cathinone-treated group exhibited increased osteoblast and osteoclast surfaces compared to the plant extract treatment. The study concluded that the plant extract and cathinone treatment are associated with minimal fat steatosis in the liver of obese mice probably by upregulation of anti-inflammatory adiponectin gene expression in adipose tissue. At the concentration of 200 mg/kg, the plant extract showed a protective effect on bone loss compared to orlistat, while cathinone promotes bone formation and resorption by a centrally mediated mechanism.

Keywords Benzoylethanamine · Bone mass · Inflammatory Biomarker · Khat · Monoamine alkaloid · Obese mice

Introduction

There is an increasing number of research findings which considered obesity as a state of low-grade inflammation (Ramos et al. 2003; Wellen and Hotamisligil 2003; Guo et al. 2019). The systemic inflammatory process affects adipose tissue, liver (Balistreri et al. 2010), and bone (Halade et al. 2011). Animal and human studies showed that overloading of adipocytes by fat lead to a state of adipocytes hypertrophy which result in cellular stress and death (Kotzbeck et al. 2018). This adaptive cellular response attracts macrophages to the inflamed area and increases systemic inflammatory mediators, such as tumor necrosis factor (TNF), interleukin 6 (IL-6), and resistin, and inversely reduces adiponectin release. A similar deposition of the fat droplet within hepatocytes (Coenen and Hasty 2007; Picchi et al. 2011) lead to a well specific inflammatory disease known as non-alcoholic fatty liver disease.

Bone research in animal and human have indicated differences related to obesity and its impact on bone mass. In young mice fed on a high-fat diet (HFD), trabecular bone and mineral content were reduced compared to young mice fed on a standard diet (Lu et al. 2013), while in adult mice HFD initially caused positive bone remodeling with high bone mass due to weight loading on skeleton followed by negative bone remodeling associated with metabolic impairment

✉ Mustafa Ahmed Alshagga
mustafa.al-shagga@nottingham.edu.my

1 Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

2 Division of Biomedical Sciences, School of Pharmacy, Faculty of Science & Engineering, University of Nottingham Malaysia Campus, Jalan Broga, 43500 Semenyih, Selangor, Malaysia

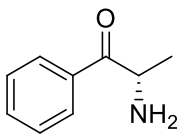
3 Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia

4 School of Clinical Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, 246 Clayton Road, Clayton, VIC 3168, Australia

and increase adiposity of bone marrow (Lecka-Czernik et al. 2015). Unlike adipose tissue, bone marrow adiposity does not trigger proinflammatory mediators; however, progenitor stem cells in bone differentiated into adipocytes, which decreases osteoblast formation (Tencerova et al. 2018), and subsequently, bone formation is affected. In addition, human studies showed that obesity might prevent osteoporosis (Maïmoun et al. 2016) as weight strengthens bearing bone mass, and increased conversion of androgens into bone-persevering oestrogens in adipose tissue (Gómez-Ambrosi et al. 2008). Orlistat is a gastric and pancreatic lipase inhibitor used for weight loss in obese patients (Drew et al. 2007). Theoretically, inhibition of lipid absorption may affect vitamin D and calcium absorption; hence, relative bone resorption could be an adverse effect of orlistat. However, this adverse effect is still unclear in humans (Drew et al. 2007).

Catha edulis (Vahl) Forssk. ex Endl., Celastraceae, known as khat or qat, is an evergreen flowering plant which grows in the eastern province that encompasses Southern Africa to the Arabian Peninsula (Lim et. al 2021). The primary alkaloid of this plant is cathinone (**1**), *S*-(-)α-aminopropiophenone, which is a monoamine alkaloid chemically related to amphetamines, such as ephedrine (**2**) which is normally found in plants of the *Ephedra* genus. Khat has antiobesogenic and appetite suppressant properties (Alshagga et al. 2016). Although khat has shown anti-inflammatory and antioxidant properties (Vinokur et al. 2008), nevertheless, there is a shred of accumulating evidence about it associations to chronic liver disease (Orlien et al. 2018).

Although our recent study showed khat extract or cathinone was associated with upregulation of lipolytic genes in adipose tissue (Alshagga et al. 2020), no study has been done to examine the effect of khat on bone structure and obesity-induced inflammatory process. This study aimed to investigate khat extracts and its bioactive compound cathinone on liver and bone histomorphological parameters, and gene expression level of adipokines in white adipose tissue and hypothalamus of obese male mice (C57Bl/6J).



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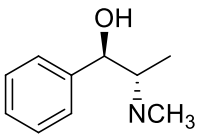

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Materials and Method

Plant Material

Fresh leaves and small stems of *Catha edulis* (Vahl) Forssk. ex Endl., Celastraceae, were harvested from Qrateel, Sana'a, Yemen (latitude 15.42506° or 15° 25′ 30″ north, longitudinal 44.05081° or 44° 3′ 3″ east) (Source: https://mapcarta.com/12493658)) and transported to Sana'a University. The plant materials were identified by a botanist (Dr. Abdul Wali Al Khulaidi) and given a voucher specimen (CAED/2015/110), which was then deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, Sana'a University.

Extraction and Cathinone Characterization

The plant material was dried dried in the dark for 10–14 days, blended and sifted into fine powder (100 g). Ethanol 95% was added into the khat powder following a proportion of 10 ml/g of powdered khat and left at room temperature for 48 h. The khat-ethanol mixture was filtered through cotton wool plug using a filter funnel followed by second filtration using filter paper. Ethanol was evaporated under low pressure using a rotary evaporator (Buchi, type R-215, Rotavapor, Switzerland). The evaporated mixture was in the form of crude dried extract (yield 19%), which was then stored at −20 °C until used. The yield of the cathinone (**1**) was measured by HPLC analysis. Separation of cathinone was achieved on a C-18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of CH_3CN/H_2O injected in 10 µl volume and was detected by a PDA detector at 330 nm. The concentration of cathinone (Rt 4.9 min) was determined by a curve of serial dilutions of standard cathinone, as described previously (Alshagga et al. 2020).

Animals

Five groups of adult mice (C57BL/6J) (n = 6 per group) were fed on the adjusted high caloric diet (18.4% protein, 23.3% carbohydrates, and 60.3% fat) obtained from Harlan Laboratories (Madison, USA) for 8 weeks as described previously (Alshagga et al. 2020). The rats were then maintained on HFD, and treatment for another 8 weeks as follows: HFD without treatment as a negative control group, HFD and orlistat 15 mg/kg as positive control (HFDO) group, HFD and khat extract 200 mg/kg as low-dose group (HFDK200), HFD and khat extract 400 mg/kg as high-dose group (HFDK400), and the last group, HFD and cathinone 3.2 mg/kg (HFDCAT) group. All mice were allowed free access to food and water *ad libitum* and treatments were carried out once daily by gastric gavage. At the end of the experiment, animals were sacrificed with

ketamine (100 mg/kg) and xylazine (10 mg/kg), and blood samples were collected using intracardiac puncture. Liver, femur, and brain were harvested.

Inflammation Biomarkers

Serum samples (40 µl) were separated and kept at −80 °C for hormone multiplex assay. The metabolic biomarker resistin, C-peptide, monocyte chemoattractant protein (MCP-1), interleukin 6 (IL-6), and tissue necrosis factor-alpha (TNF-α) were measured by mouse metabolic magnetic bead panel kit (MMHMAG-44K MILLIPLEX® MAP, Merck Millipore, Billerica, MA, USA). Preparation of samples was carried out according to the manufacturer's protocol. Serum adiponectin was measured by immunoassay method using mouse adiponectin immunoassay kit (SPI BIO, France). Liver enzymes (alanine and aspartate transaminases) analysis was carried out by automatic biochemical analyzer (Hitachi 902).

Mouse Femur

Femora were collected and rinsed in neutral formalin and EDTA for fixation after all soft tissues were cleaned off. After 4 weeks, a sagittal section of the left femur was carried out. One part was processed for decalcification method to measure structural and static histomorphological parameters. Briefly, the part of the femur was washed with tap water overnight, treated with ascending alcohol concentrations, and rinsed in toluene overnight, and paraffin liquid was added to form the wax cubes and sectioned (at 6 µm) thickness. Slides were then left to dry before staining with hematoxylin and eosin (H&E). Measurements of bone volume, bone surface area, and osteoblast were conducted using Weibel grid protocol as described in Saif et al. (2016).

Mouse Liver

The fresh liver was weighted, and then samples were fixed in 10% buffered formalin, then the processed sections (Leica TP-1020-1-1) were embedded in paraffin wax and cooled overnight. The transparent paraffin blocks were trimmed (3–4 µm) using a rotatory microtome (Leica RM 2035). The slides were stained by H&E stain, as stated in the protocol (Bancroft et al. 2018).

PCR Assay

The method was previously described in detail (Alshagga et al. 2020). RNA was extracted from epididymal adipose tissue and hypothalamus samples by TRIzol™ solution (Thermofisher Scientific, USA) followed by RNeasy plus mini kit (Qiagen, USA) and stored at −80°C until further use. For PCR array, 10 genes were customized for adipose tissue gene expression. Four genes were related to the serum biomarkers (adiponectin, IL-6. TNF, and resistin) and 6 genes in signaling inflammatory pathways. Four genes expressed in the hypothalamus were selected, two to measure hypothalamic inflammation (IL-6 and TNF) and two in signaling pathway. Inflammatory adipose tissue and hypothalamus genes are listed in Table S1. Three housekeeping genes were used in every reaction: glyceraldehyde-3-phosphate dehydrogenase, beta-actin, and hypoxanthine-guanine phosphoribosyltransferase.

Statistical Analysis

Data were expressed as mean ± SEM. One-way ANOVA was used to compare treatment groups (GraphPad Prism version 7.03). Post hoc Tukey test was used for differences among groups. All analyses employed a significance level (p-value) of 0.05. Quantitative RT-PCR array data were expressed as relative fold change of the genes in treated groups compared to the same genes in HFD control as reference. The analysis was done according to manufacturer's instruction using an integrated web tool (https://www.qiagen.com/my/shop/genes-and-pathways/data-analysis-center-overview-page/). The analysis used Student's t-test comparison of each treatment group to the control group by testing the mean ΔΔCt of each group. Fold change of 2 (up or down) was used as a cutoff point with p-value <0.05 considered a significant difference in gene expression analysis.

Results and Discussion

Liver Histology

Fatty liver changes, such as the presence of macrovascular and microvascular steatosis, were observed in the liver of control group (Fig. 1A). The accumulation of fat vacuoles in low-dose khat extract (Fig. 1C) and orlistat (Fig. 1B) showed a moderate reduction in fat vacuoles, while high-dose khat extract (Fig. 1D) and cathinone (Fig. 1E) groups showed minimal areas of fatty changes. Liver mass was reduced insignificantly in orlistat and khat (200 mg/kg) groups, while khat (400 mg/kg) and cathinone-treated groups showed a significant (p <0.05) reduction of liver mass and alanine transferase compared to control (Fig. 2).

Bone Histology and Parameters

The bone cells, eroded bones, osteoid, and marrow adipocytes are illustrated in the bone histology images (Fig. 3)

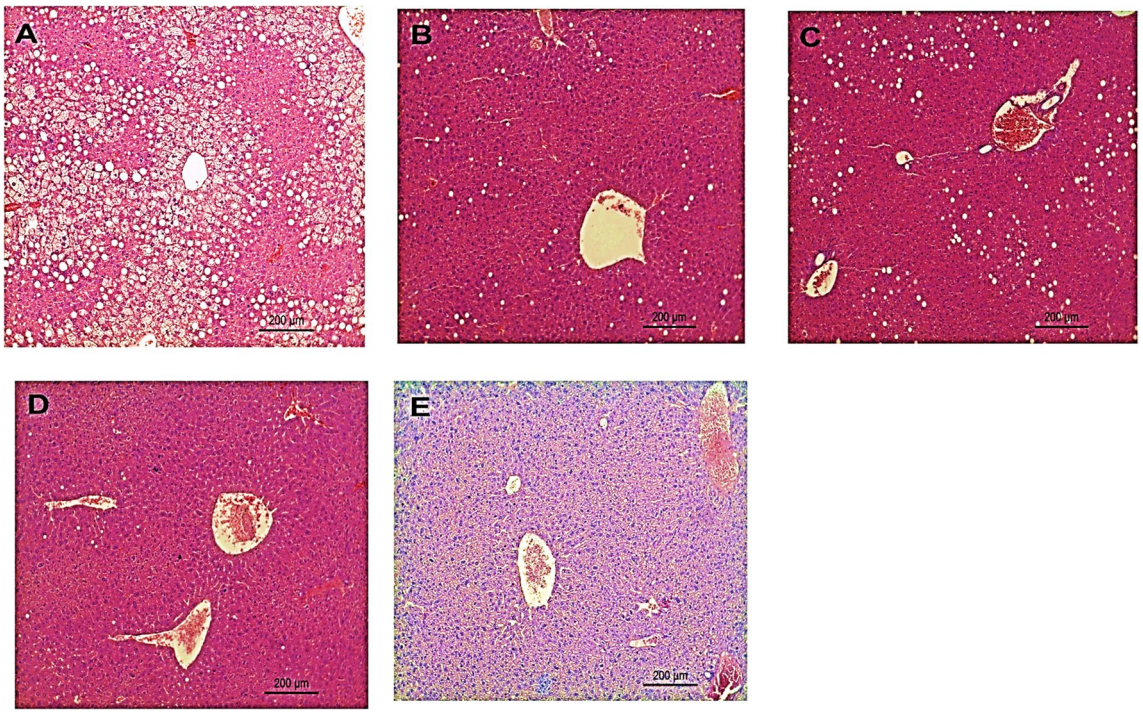

Fig. 1 Liver histology shows the scattered fat vacuoles after treatment. **A** HFD control, **B** HFD + orlistat (15 mg/kg), **C** HFD + khat (*Catha edulis*) (200 mg/kg), **D** HFD + khat (400 mg/kg), and **E** HFD + cathinone (3.2 mg/kg)

while structural and static bone parameters are shown in Fig. 4. The static bone histomorphological findings of HFD-treated groups showed that HFDK200 and HFDCAT treatment were associated with significantly (p <0.01) higher bone surface, bone volume, and lower trabecular separation compared to the HFDC group as shown in Fig. 4. The low-dose khat extract was also associated with less eroded surfaces (p <0.01) in comparison to orlistat- and cathinone-treated groups. An interesting finding in this study, the cathinone treated-group, was associated with increased osteoblast and osteoclast surfaces compared to khat extract treatment (p <0.05). However, no significant difference was observed as compared to orlistat and control groups (Fig. 4).

Inflammatory Biomarker and Gene Expression

A significant elevation of serum adiponectin was observed in all treated groups compared to control littermates (Fig. 5) which probably matched well with significant upregulation of adiponectin gene (Adipoq) in adipose tissue (Table 1).

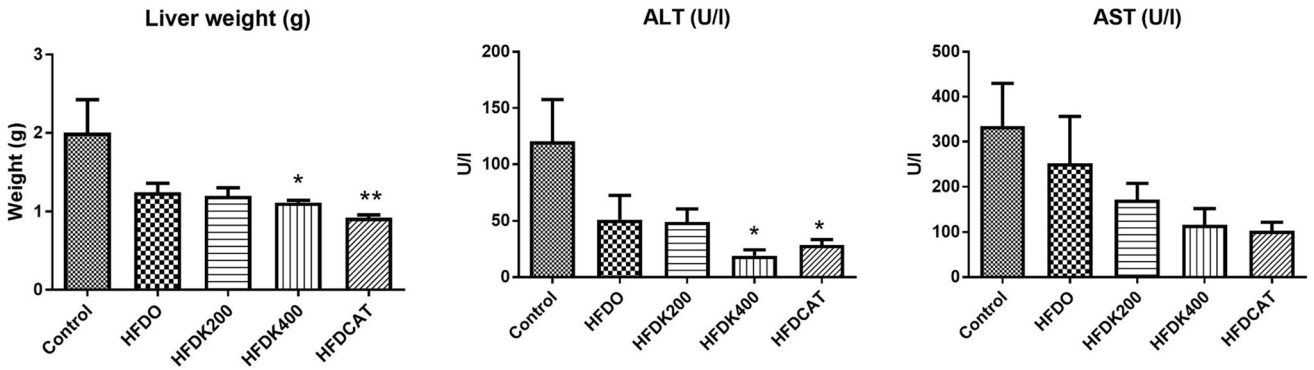

Fig. 2 Comparison of liver mass (g) and transaminases (U). n=6; *p-value < 0.05, **p-value < 0.01 compared to control. Data are expressed as mean ± SEM. ALT, alanine transaminase; AST, aspartate transaminase; control, high-fat diet control; HFDO, high-fat diet + orlistat (15 mg/kg); HFDK200, high-fat diet + khat (*Catha edulis*) (200 mg/kg); HFDK400, high-fat diet + khat (400 mg/kg); and HFDCAT, high-fat diet + cathinone (3.2 mg/kg)

Inversely, serum resistin showed a marked reduction in khat- and cathinone-treated groups compared to control (Fig. 5); nonetheless, resistin (Retn) gene expression showed non-significant difference among groups.

Cathinone-treated group showed a significant low serum level interleukin-6 (IL-6) compared to the control mice (Fig. 5). The gene expression of IL-6 in adipose tissue showed non-significant downregulation and surprisingly significant upregulated hypothalamic IL-6 in low-dose khat (200 mg/kg) group (Table 1). Downstream signaling genes for the proinflammatory mediator (JAK2, STAT3, and NF-kB) did not show a significant result.

The effect of khat or its active alkaloid cathinone have not been studied in the liver or bone histology of obese mice to date. This study shows that khat or cathinone treatment is associated with a protective effect against liver fatty changes due to HFD and able to elevate anti-inflammatory adiponectin in a similar way to orlistat treatment. The findings on bone indicate that a low khat dose showed less osteoporotic changes compared to control and other treated groups, while cathinone associated with an increase in the bone remodeling of the obese mice. In this study, khat (400 mg/kg) and cathinone (3.2 mg/kg) treatment reduced liver mass significantly compared to control littermates. Doses of orlistat, khat, and cathinone were chosen based on a previously reported study (Alshagga et al. 2020; Xia et al. 2020). In another experiment on C57BL/6J mice fed on HFD, the fat mass and liver mass significantly increased after 12 weeks (Griffin et al. 2019). However, when mice switched to standard diet for 8 weeks, mice were returned to average weight; fat and liver masses were also reduced after dietary weight loss intervention (Griffin et al. 2019). In this study, fatty liver changes were marked in mice fed on HFD. The effectiveness of orlistat to improve fatty liver in clinical trials had been reported (Zelber-Sagi et al. 2006; Khan et al. 2017) which was consistent with the results of this study. Khat extract (200 mg/kg) has produced a very similar morphological protective effect as orlistat, while a high khat dose (400 mg/kg)- and cathinone (3.2 mg/kg)-treated groups showed more effective protection against development of fatty liver. A 6-week khat treatment study and a 13-week khat treatment study demonstrated the absence of hepatotoxicity in khat-treated rats as well as revealed antioxidant property for the extract (Al-Zubairi et al. 2008a, 2008b). The published studies attributed the antioxidant effect to be due to the polyphenol content in khat leaves. However, in our liver, histology results showed pure cathinone (3.2 mg/kg) treatment has associated with minimal fatty changes. Cathinone effect on liver had not been studied previously; therefore, it would be difficult to validate whether it is a direct effect of cathinone on hepatocyte or indirect effect due to reduction in body weight and serum triacylglycerides.

Cathinone and amphetamine are linked to appetite suppressing and weight loss (Lemieux et al. 2015). Brain catecholamines are needed to trigger anorexigenic activity of amphetamine and other structurally related compounds.

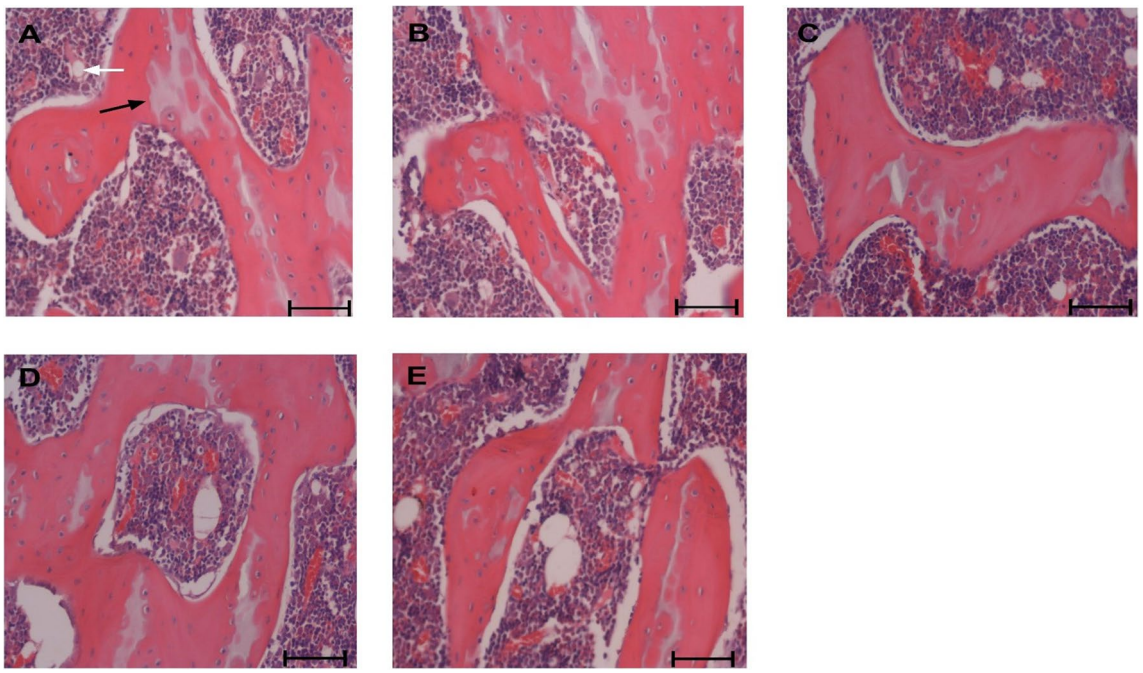

Fig. 3 Bone histology shows eroded bone (black arrow) and marrow adipocyte (white arrow). **A** HFD control, **B** HFD + orlistat (15 mg/kg), **C** HFD + khat (*Catha edulis*) (200 mg/kg), **D** HFD + khat (400 mg/kg), and **E** HFD + cathinone (3.2 mg/kg)

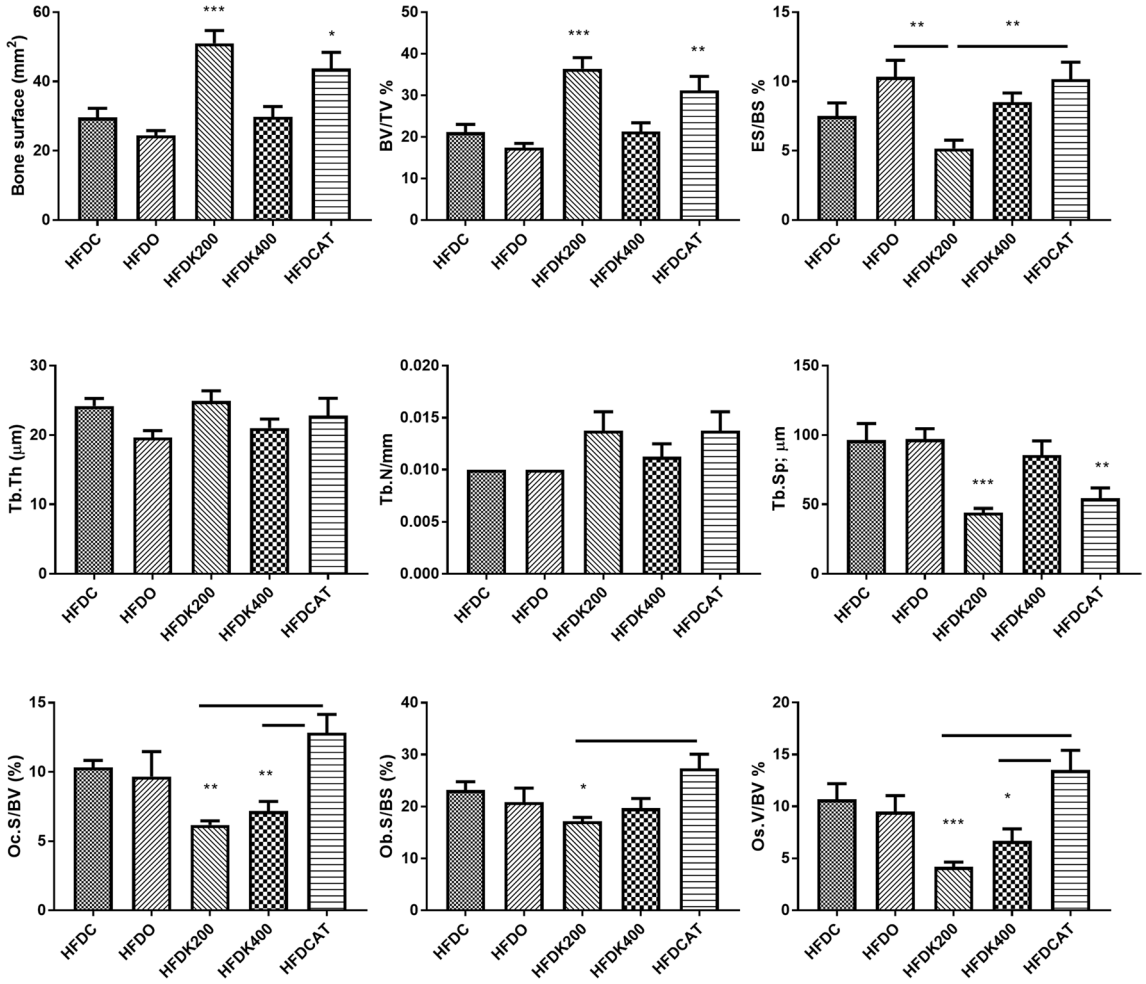

Fig. 4 Comparison of bone histomorphological structural parameters. Data are expressed as mean ± SEM; n=6; *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.0001 multiple comparisons. HFD, high-fat diet control; HFDO, high-fat diet + orlistat (15 mg/kg); HFDK200, high-fat diet + khat (*Catha edulis*) (200 mg/kg); HFDK400, high-fat diet + khat (400 mg/kg); and HFDCAT, high-fat diet + cathinone (3.2 mg/kg); BV/TV, bone volume/tissue volume; ES/BS, eroded surface/bone surface; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Oc.S/BV, osteoclast surface/bone volume; Ob.S/BS, osteoblast surface/bone surface; Os.V/BV, osteoclast volume/bone volume

Amphetamine and methamphetamine mediate the release of dopamine (Ferrucci et al. 2019). Amphetamine and structurally related compounds inhibit feeding through dopamine level elevation (West et al. 2019). The mechanism of cathinone on neurotransmission is via mediation of dopamine release and dopamine reuptake inhibition which is similar to mechanism exert by amphetamine (Dasgupta 2019). Studies reported decreased dopamine levels and lower dopamine receptor availability or affinity is associated to binge eating in rat model and human subjects (Yu et al. 2022). Moreover, there is a positive relationship between polymorphism of dopamine-regulating genes and higher levels of binge eating, where individuals with COMT Val/Val (catechol-*O*-methyltransferase), homozygous for valine form (Val/Val) genotype, degrades dopamine faster leading to lower dopamine levels and subsequently higher levels of binge eating (Yu et al. 2022). Appetite suppressants including amphetamine and phenethylamines exert their anorectic effects via D1- and D2-like receptors (Kalyanasundar et al. 2020).

Both male and female C57BL/6 mice were subjected to nutritional and hormonal changes that may produce different energy homeostasis and metabolic outcomes (de Souza et al. 2022). Male C57BL/6 mice were selected in this study as the mice lost more weight and lean body mass in response to food restriction as compared to female mice (de Souza et al. 2022). Male mice also gained more fat and exhibited lower energy expenditure and glycemia after refeeding while female mice exhibited higher protection against diet-induced obesity (de Souza et al. 2022). The inflammatory response of obesity is characterized by an increment of macrophages' number in adipose tissue and liver (Griffin et al. 2019). Discontinuation of high-fat diet reduced macrophages number

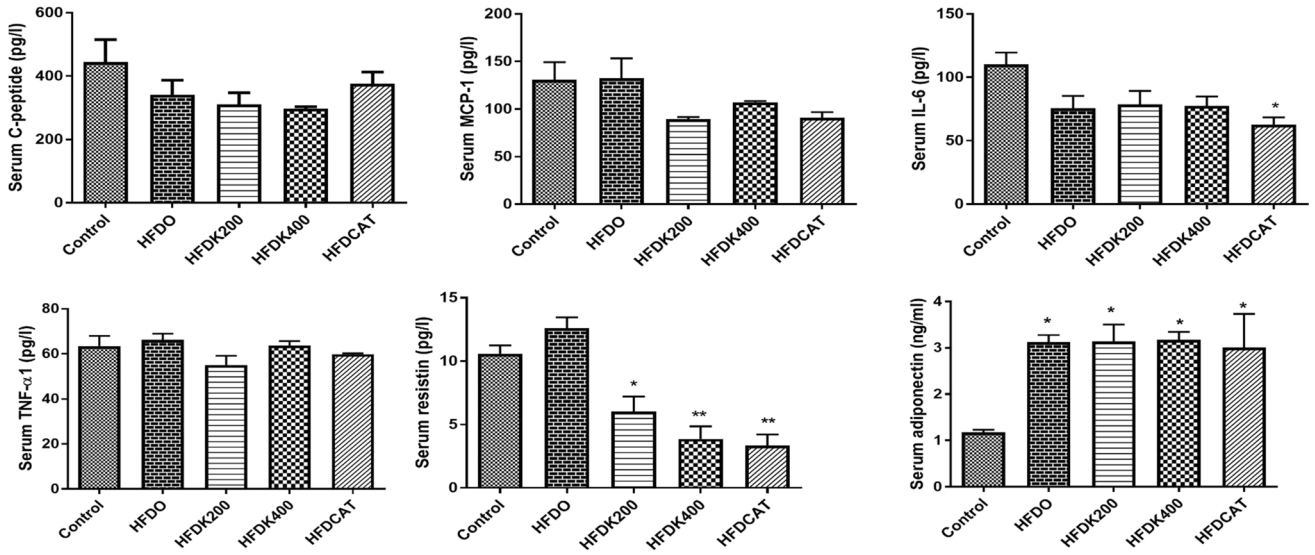

Fig. 5 Comparison of serum inflammatory biomarkers. Data are expressed as mean ± SEM; n=6; *p-value < 0.05, **p-value < 0.01 compared to control. Control, high-fat diet + vehicle; HFDO, high-fat diet + orlistat (15 mg/kg); HFDK200, high-fat diet + khat (*Catha edulis*) (200 mg/kg); HFDK400, high-fat diet + khat (400 mg/kg); and HFDCAT, high-fat diet + cathinone (3.2 mg/kg)

in male mice; however, the inflammatory response was not entirely resolved in male compared to female mice (Griffin et al. 2019). This finding in Griffin et al. (2019) probably could explain why our male mice in this study did not show a significant difference in serum C-peptide, MCP-1, and TNF, and to some extent on IL-6 after treatment with khat extract and cathinone for 8 weeks. In addition, the central upregulation of IL-6 in khat treated groups in this study possibly indicate the presence of inflammatory process caused by HFD (Bobbo et al. 2019). The effect of HFD on male C57BL/6J bone was suggested to have two phases. Prolonged overconsumption of HFD leads to HFD-induced obesity and linked to cytokine production by non-neuronal cells within the hypothalamus, triggering metabolic inflammation as observed in obesity (Ullah et al. 2021).

The early phase of the metabolic outcomes is characterized by an increase in bone mass due to intensification in mechanical loading, an increment of insulin and anabolic adipokines (Lecka-Czernik et al. 2015), followed by a detrimental second phase of increased marrow adiposity and less osteoblast formation. These bone changes are inconsistent with C57BL/6 female mice fed on HFD that revealed improved trabecular number and thickness, and reduced trabecular separation compared to control mice fed on a normal diet (Peng et al. 2014). The gender difference may be attributed to the estrogenic protective effect on bone, which is well documented.

Our current findings reported protective effect of the low dose of crude khat extract that significantly reduced trabecular separation and eroded bone surfaces; paradoxically, a high khat dosage may deteriorate bone structure. In a human male study, khat chewers have shown higher oestradiol and testosterone plasma levels compared to non-chewers (Al-Ghamdi 2012) and associated with raised calcium level (El-Sayed and Amin 2012). Nonetheless, the former study concluded the effect due to hormonal imbalance, while the latter attributed the effect to high content of calcium in khat leaves, while in animal studies, testosterone level is increased in low doses of khat extract treatment (Mwenda et al. 2006; Mohammed and Engidawork 2011), while a high-dose khat extract reduced serum testosterone (Mohammed and Engidawork 2011). Since testosterone is converted to estradiol peripherally (Yibrah et al. 2019), so we may explain the protective low dose of khat (200 mg/kg) on bone is by a testosterone increment. In contrast, the active alkaloid of khat, cathinone, enhanced both osteoblast and osteoclast as this study revealed, which indicates active bone remodeling in mice compared to khat extract–treated groups. The mechanism for this finding was probably mediated by a central regulation. It has been shown that both hypothalamic peptide, CART and NPY, expressions promoted bone formation, whereas resorption was mainly mediated by NPY (Idelevich et al. 2020). Our previous findings supported this explanation to some extent as we found cathinone upregulates hypothalamic AgRP and CART, but not NPY of obese mice (Alshagga et al. 2020).

The upregulation of adiponectin did not induce a change in TNF expression in adipose tissue (Table 1). The elevated adiponectin level also could explain the reduction of fat deposition in liver (Liu et al. 2017) which was similar to findings on rats (Milan et al. 2002). Milan et al. (2002) found a significantly downregulated resistin gene in adipose tissue

Table 1 Effect of orlistat, khat (*Catha edulis*) extract, and cathinone on adipose tissue and hypothalamus gene expressions compared to high-fat diet control group

Inflammatory gene expression in mice adipose tissue				HFD + orlistat (15 mg/kg)		HFD + khat (200 mg/kg)		HFD + khat (400 mg/kg)		HFD + cathinone (3.2 mg/kg)	
	Unigene	Refseq	Symbol	Fold regulation	*P*-value	Fold regulation	*P*-value	Fold regulation	*P*-value	Fold regulation	*P*-value
Leptin	Mm.277072	NM_008493	Lep	1.808	0.511	3.746	0.842	2.837	0.986	2.771	0.910
Adiponectin, C1Q and collagen domain containing	Mm.3969	NM_009605	Adipoq	53.520	0.341	**51.524**	**0.020**	**67.844**	**0.003**	44.242	0.088
Complement factor D (adipsin)	Mm.4407	NM_013459	Cfd	7.602	0.385	13.662	0.418	59.021	0.961	81.306	0.322
Resistin	Mm.1181	NM_022984	Retn	0.423	0.348	2.107	0.798	1.486	0.670	0.499	0.629
Interleukin 6	Mm.1019	NM_001314054	Il6	0.08	0.211	0.338	0.272	0.196	0.232	0.122	0.216
Tumor necrosis factor	Mm.1293	NM_013693	Tnf	0.011	0.356	0.011	0.356	0.003	0.356	0.003	0.356
Chemokine (C-C motif) ligand 2	Mm.290320	NM_011333	Ccl2	1.631	0.401	2.031	0.414	0.620	0.387	1.014	0.382
Inhibitor of kappaB kinase beta	Mm.277886	NM_010546	Ikbkb	1.147	0.529	1.998	0.878	2.019	0.873	2.324	0.593
Janus kinase 2	Mm.275839	NM_008413	Jak2	0.464	0.389	0.777	0.449	0.673	0.411	1.003	0.559
Signal transducer and activator of transcription 3	Mm.473190	NM_011486	Stat3	0.530	0.691	1.204	0.775	1.753	0.567	1.679	0.636
Inflammatory gene expression in mice hypothalamus	**Unigene**	**Refseq**	Symbol	Fold regulation	*P*-value	Fold regulation	*P*-value	Fold regulation	*P*-value	Fold regulation	*P*-value
Interleukin 6	Mm.1019	NM_001314054	Il6	1.275	0.283	**3.004**	**0.045**	1.752	0.151	8.572	0.069
Tumor necrosis factor	Mm.1293	NM_013693	Tnf	1.460	0.474	0.922	0.541	0.529	0.199	1.146	0.794
Inhibitor of kappaB kinase beta	Mm.277886	NM_010546	Ikbkb	1.081	0.553	1.303	0.231	1.135	0.174	1.395	0.277
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	Mm.170515	NM_010907	Nfkbia	1.454	0.026	0.988	0.926	0.684	0.070	1.461	0.238

* All *p*-values (**bold**) are obtained by Student's *t*-test comparing gene expression in each treated group to HFD group gene expression (According to the manufacturer's instruction using web integrated tool (https://www.qiagen.com/my/shop/genes-and-pathways/data-analysis-center-overview-page/)). #Fold change compared to control HFD group = 1

which is not the case in our study. However, serum resistin levels were lower in khat- and cathinone-treated groups compared to control mice which is inconsistent finding to human study (El-Sayed and Amin 2012). The hormonal discrepancy in human and animal studies had been reported on the pharmacological effect of khat (Engidawork 2017).

Conclusion

Khat or cathinone treatment is associated with minimal fat steatosis in the liver of obese mice; this is explained by upregulation of anti-inflammatory adiponectin gene expression in adipose tissue. The low dose of khat (200 mg/kg) showed a protective effect on bone loss and strengthened bone surface and volume compared to high-dose khat (400 mg/kg), while cathinone promotes bone formation.

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Author Contribution MAA, MA Alshawsh, NM, YP, SYML, and ZM designed the study. MAA and AS performed the experiment on obese mice. MAA and NM performed bone analysis. MAA, MA Alshawsh, and NM analyzed the data. MAA drafted the manuscript. MA Alshawsh, NM, YP, SYML, and ZM revised the paper. All authors read and accept the final version of the manuscript.

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Declarations

Ethical Approval The Faculty of Medicine approved all protocols in this section, the University of Malaya's Animal Ethics Committee approved the protocol with ethical approval number (2014-08-05/PHAR/R/MAMA). All animals were handled as instructed in the "Guide of Care and Use of Laboratory Animals" criteria of the National Academy of Sciences (Academy and of Sciences , 2011).

Conflict of Interest The authors declare no competing interests.

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