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A hydroalcoholic blackberry leaf extract improved glucose tolerance and body composition and attenuated depression-like symptoms in ovariectomized rats.

O extrato hidroalcóolico das folhas de amora preta melhorou a tolerância à glicose, a composição corporal e atenuou sintomas de depressão em ratas ovariectomizadas.

El extracto hidroalcohólico de hojas de mora mejoró la tolerancia a la glucosa, la composición corporal y atenuó los síntomas de depresión en ratas ovariectomizadas.

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Abstract

Blackberry leaves (*Morus nigra*) are used in menopause to relieve hot flashes and night sweats and have been investigated in metabolic disorders. However, few studies investigated its effects on metabolic and behavioral disorders when associated with menopause. This study evaluated the effects of a hydroalcoholic extract of *M. nigra* leaves (MN) on body composition, glucose tolerance, and depression and anxiety-like behaviors in ovariectomized rats. For this purpose, 36 female Wistar rats were divided into groups: C – control; COvx – ovariectomized; MNOvx – ovariectomized and given MN at 400 $mg.kg^{-1}$ body mass/day. Body composition was assessed by DEXA, glucose tolerance by the oral glucose tolerance test, and depression and anxiety-like behaviors by the forced swimming and elevated plus maze tests, respectively. Food intake and body mass gain were higher for MNOvx, followed by COvx and C ($p<0.05$). The MNOvx group gained less body fat than COvx ($p<0.05$) and was more glucose tolerant compared to C and COvx ($p<0.05$). In the elevated plus maze, COvx and MNOvx remained for less time in the open arms and longer in the closed arms than C ($p<0.05$). In forced swimming, immobility time was higher for COvx, followed by C and MNOvx ($p<0.05$). The MN protected ovariectomized rats from body fat gain, improved glucose tolerance, and attenuated depression-like behavior. Phytoestrogens and other bioactive compounds in MN may be involved in these effects.

Keywords: Body composition; Menopause; *Morus nigra*; Glucose tolerance; Depression; Anxiety.

Resumo
As folhas da amoreira preta (*Morus nigra*) são utilizadas na menopausa para alívio de alguns sintomas e vem sendo investigadas em distúrbios metabólicos. Entretanto, poucos estudos investigaram seus efeitos em disfunções metabólicas e comportamentais associadas à menopausa. Nosso objetivo foi avaliar os efeitos do extrato hidroalcóolico de folhas da *M. nigra* (EA) na composição corporal, tolerância à glicose e nos comportamentos semelhantes à depressão e ansiedade em ratas ovariectomizadas. Para tal, 36 ratas *Wistar* foram distribuídas nos grupos: C – controle; COvx – ovariectomizado; EAOvx – ovariectomizado e suplementado com EA (400 mg.kg^{-1} massa corporal/dia). Avaliou-se a composição corporal por DEXA, a tolerância à glicose pelo teste de tolerância oral à glicose e os comportamentos semelhantes a depressão e ansiedade pelos testes nado forçado e labirinto em cruz elevada, respectivamente. A ingestão alimentar e o ganho de massa corporal foram maiores para EAOvx, seguido por COvx e por último por C (p<0,05). O grupo EAOvx ganhou menos gordura corporal do que o COvx (p<0,05) e foi mais tolerante à glicose em comparação ao C e COvx (p<0,05). No labirinto em cruz elevada, COvx e EAOvx permaneceram por menos tempo nos braços abertos e mais tempo nos braços fechados que os C (p<0,05). No nado forçado, a imobilidade foi maior para COvx, seguido pelo C e EAOvx (p<0,05). O EA protegeu as ratas ovariectomizadas do ganho de gordura corporal, restabeleceu a tolerância à glicose e atenuou o comportamento semelhante à depressão. Fitoestrogênios e outros compostos bioativos do EA podem estar envolvidos nesses efeitos.
Palavras-chave: Composição corporal; Menopausa. *Morus nigra;* Tolerância à glicose; Depressão; Ansiedade.

Resumen
Pocos estudios han investigado los efectos de las hojas de mora (*Morus nigra*) sobre los trastornos metabólicos y conductuales asociados con la menopausia. Nuestro objetivo fue evaluar los efectos del extracto hidroalcohólico de hojas de *M. nigra* (EA) sobre la composición corporal, la tolerancia a la glucosa y los comportamientos depresivos y ansiosos en ratas hembra ovariectomizadas. Para ello, 36 ratas Wistar hembras fueron divididas en grupos: C – control; COvx – ovariectomizados; EAOvx – ovariectomizados y suplementados con EA (400 mg.kg-1 de masa corporal/día). La composición corporal se evaluó mediante DEXA, la tolerancia a la glucosa mediante la prueba oral de tolerancia a la glucosa y las conductas de depresión y ansiedad mediante las pruebas de natación forzada y laberinto en cruz elevado, respectivamente. La ingesta de alimentos y la ganancia de masa corporal fueron mayores para EAOvx, seguida de COvx y finalmente C (p<0,05). El grupo EAOvx ganó menos grasa corporal que COvx (p<0,05) y fue más tolerante a la glucosa en comparación con C y COvx (p<0,05). En el laberinto en cruz elevado, COvx y EAOvx permanecieron menos tiempo en los brazos abiertos y más tiempo en los brazos cerrados que C (p<0,05). En natación forzada, la inmovilidad fue mayor para COvx, seguida de C y EAOvx (p<0,05). EA protegió a las ratas ovariectomizadas del aumento de grasa corporal, restableció la tolerancia a la glucosa y atenuó el comportamiento similar a la depresión. Los fitoestrógenos y otros compuestos bioactivos en EA pueden estar involucrados en estos efectos.
Palabras clave: Composición corporal; Menopausia; *Morus nigra*; Tolerancia a la glucosa; Depresión; Ansiedad.

1. Introduction

Menopause is generally defined as the permanent cessation of menstruation. It is classified into premenopausal, perimenopausal, and postmenopausal stages, defined based on endocrine status and regularity of menstrual bleeding pattern (Harlow et al., 2012). Menopause manifests in women aged between 46 and 52 years, signaling the end of reproductive life due to ovarian follicular exhaustion (Voedisch et al., 2021). The absence of complete menstruation defines this condition as being considered early before age 40 and late after age 55. Menopause can also occur due to surgical procedures, chemotherapy, or radiation (Edwards & Li, 2013). Estimates indicate that by the year 2030, approximately 1.2 billion women will be in the process of menopause or post-menopause, with 47 million new entrants in this period each year (Johnson et al., 2019).

During perimenopause, there is a reduction in the number of ovarian follicles, which reduces the inhibin-B protein release, increasing the anterior pituitary stimulus for follicle-stimulating hormone (FSH) production, which at last, stimulates the estradiol (E2) ovarian production. As long as women undergo this transition, the ovarian follicle supply becomes critically low, and ovaries can no longer respond to increased FSH signaling with consistent E2 production. Eventually, all functional follicles are lost, and E2 production by the ovaries ceases, accompanied by a steady state of high FSH production (Marlatt et al., 2022). Due to hormonal changes, some undesirable symptoms manifest in 80% of women during perimenopause, such as hot flashes, night sweats, changes in mood and sleep quality, sexual dysfunction, and fat mass gain. These symptoms affect the quality of life and increase the risk for cardiometabolic diseases (Monteleone et al., 2018).

Women have been shown to gain approximately 2 to 3 kg of fat mass during perimenopause (Al-Safi & Polotsky, 2015;

Lizcano & Guzmán, 2014; Marlatt et al., 2020; Shifren et al., 2014). These changes are associated with changes in energy expenditure and intake that favor positive energy balance, promoting body mass and fat gain, especially abdominal (Marlatt et al., 2022). Furthermore, it has also been suggested that E2 protects from central body fat accumulation (Bruns & Kemnitz, 2004), which in perimenopause could also favor fat accumulation in this region. Abdominal fat accumulation is associated with more severe vasomotor symptoms (hot flashes and night sweats) and insomnia (Thurston et al., 2008, 2009), increased fatigue, and reduced quality of life (Jones & Sutton, 2008; Wadden et al., 2006). In addition, it promotes a more pro-inflammatory environment due to an increase in the pro-inflammatory cytokines (TNF-α and IL-6, for example) and a reduction in anti-inflammatory ones (IL-10, for example) production (Sinatora et al., 2022), which also contributes to cardiometabolic disorders (Jaballah et al., 2021).

The changes in E2 production also directly affect glucose homeostasis since it contributes to insulin secretion and clearance (Godsland, 2005). It also has been shown that E2 increases muscle glucose uptake by positively affecting insulin signaling pathway elements. It also reduces hepatic glucose production (Yan et al., 2019). Indeed, in perimenopause, events such as hyperglycemia, hyperinsulinemia, insulin resistance, and glucose intolerance have been reported, which also contribute to increasing cardiometabolic disease risk (de Paoli et al., 2021). Furthermore, studies show that endocrine changes (Soares, 2007) and bothersome menopausal symptoms, including hot flushes, night and day sweats, and sleeping problems, put women at a higher risk of psychological symptoms in this phase of life (Lund et al., 2018). In Western culture, menopause is also perceived as a loss of sexual attractiveness, leading to depression (Lock, 1991; Obermeyer, 2000).

Therefore, it is undeniable that menopausal symptoms and increased cardiometabolic risk harm women's health. Because of that, several pharmacological and non-pharmacological strategies are used in this period. From this perspective, hormone replacement therapy is considered the primary pharmacological strategy during this process, and different classes of drugs are used, including estrogens, progestogens, or their combinations. (Palacios et al., 2019). However, since the findings from World Health Initiative (Rossouw et al., 2002) indicated that women using hormone replacement therapy had an increased risk of invasive breast cancer, the focus has been on finding effective non-pharmacological strategies.

In this context, *M. nigra* leaves are widely used in the form of teas, especially in Japan and Korea, as a food supplement (Polumackanycz et al., 2021). Previous phytochemical studies have shown that *M. nigra* leaves contain flavonoids, stilbenes, alkaloids, and coumarins with various biological properties, such as anti-inflammatory, antioxidant, hepatoprotective, anti-tumor, and antidiabetic effects (Lim & Choi, 2019; Sokri et al., 2021; Wang et al., 2022). However, few studies still investigate *M nigra* leaves' effects on reducing risks for metabolic disorders and psychological symptoms associated with menopause. Therefore, this study aimed to evaluate the effects of a hydroalcoholic extract of *M. nigra* leaves on body composition, glucose tolerance, anxiety, and depression-like behaviors in ovariectomized rats as an experimental model of menopause.

2. Methodology

A hydroalcoholic extract of *M. nigra* leaves (MN), standardized at 40% of dry matter, was used in this study. The extracting solution was 70% alcohol. The extract was purchased from Phytoshop Panizza Fitoterápicos (São Paulo-SP) and kept under refrigeration (5 ± 2ºC) in an amber bottle until use. The extract underwent physical-chemical and microbiological quality tests, meeting the requirements of the identity and quality standards determined by the Agência Nacional de Vigilância Sanitaria - ANVISA (ANVISA, 2007).

In the bioassay, female Wistar rats (Rattus norvegicus, n = 36), approximately 90 days old, 348±20g, were maintained in a 12/12-h reverse photoperiod, at a temperature of approximately 22 - 24 ◦C, with water and food ad libitum throughout the experimental period. The experimental protocol and animal handling were approved by the Ethics Committee on the Use of Experimental Animals - CEUA, UFVJM (# 011/2020).

After one week of acclimatization, rats were randomly allocated to three experimental groups (n=12): C: control (did not undergo ovariectomy); COvx: ovariectomized control, to mimic human menopause and MNOvx: ovariectomized receiving a daily dose of MN at 400 $mg.kg^{-1}$ of body weight, similar to Júnior et al. (2017). The extract was properly homogenized in 3 grams of chow and offered daily to the MNOvx group to ensure total intake of the predefined dose. The animals were housed individually every morning and had free access to the pellet. After total ingestion, they were returned to their box of origin. The other groups underwent the same procedure but received just 3 grams of chow.

One week later, the COvx and MNOvx groups underwent bilateral ovariectomy, as described elsewhere (Moreira et al., 2005; Savergnini et al., 2012), and the experiment lasted for another eight weeks under the same conditions. Body mass and food intake were monitored daily. At the end of the protocol, it was calculated the feed efficiency ratio (FER = [body mass gain/food intake] * 100).

In the penultimate week, all rats were submitted to two behavior tests. In the elevated plus maze test, we assessed anxious behavior based on the animals' propensity for dark and protected spaces and fear of open spaces and heights, combined with their innate motivation to explore new environments (Ari et al., 2019). The maze consisted of a platform composed of two open arms (without walls) and two closed arms with high walls (each one 10.0 cm wide and 50.0 cm long and walls 40.0 cm high), connected by a central square (9 × 9 cm), elevated 53 cm from the floor. The animals were placed individually in the center of the device, facing one of the closed arms, and allowed to explore the maze. During the 5-minute sessions, the time each animal spent in the open or closed arms was determined using a USB camera connected to the Etho Vision Noldus XTV V Software 16 (Figueiredo et al., 2019). In the forced swimming test, we assessed depressive-like behavior. A pool filled with water at 23-25°C was used, and the animals were allowed to swim for 15 minutes to acclimate to the environment. Afterward, they were removed from the water, dried, and returned to their respective boxes. Twenty-four hours after habituation, they were returned to the pool and allowed to swim for 5 minutes; their performance was filmed for later analysis. After 5 minutes, the animals were removed from the water, dried, and returned to their respective cages. The videos were analyzed, and the immobility time of each animal was recorded (Slattery & Cryan, 2012).

In the last week of the protocol, all animals underwent body composition analysis by dual-energy X-ray absorptiometry (DEXA Lunar iDXA, GE. Wisconsin – U.S.A.). At the end of the same week, they were submitted to an oral glucose tolerance test, according to Fraulob et al. (2010). Blood glucose concentration was measured using a portable glucose meter (Roche, Accu-Chek Performa Nano, Rio de Janeiro, Brazil). Blood glucose concentration curves were plotted from these data over time, and the area under the curve (AUC) was calculated.

All results were expressed as means ± standard deviation. Data were previously tested to verify normality. To compare the variables between the experimental groups, we used the One-way ANOVA and Tukey test. Differences were considered significant when $p<0.05$. Statistical analyzes were performed using SigmaPlot software, version 12.

3. Results

At the beginning of the experiment, body mass (C=250.83±18.20; COvx=255.00±14.30 and MNOvx=266.67±20.04) did not differ among the experimental groups (Figure 1A). In the end, the COvx and MNOvx groups weighed equally more (302.92±17.64 and 320.83±25.57 respectively) than C (280.00±24.12; $p<0.005$, Figure 1B). However, the body mass gain was higher for the MNOvx group (54.17±13.11), followed by COvx (47.92 ±10.54) and finally by C (29.17 ±17.03; $p<0.05$; Figure 1C). Similar results were observed for food intake (C=487.58 ±31.59, COvx= 555.57 ±13.59, MNOvx= 590.19 ±46.04; $p<0.05$; Figure 1D). However, these differences were not enough to promote a different FER between the COvx and MNOvx groups (COvx= 8.63 ±1.89; MNOvx= 9.14±1.93), which had ratios equally higher than those of C (C =5.94 ±3.25; $p<0.05$, Figure 1E).

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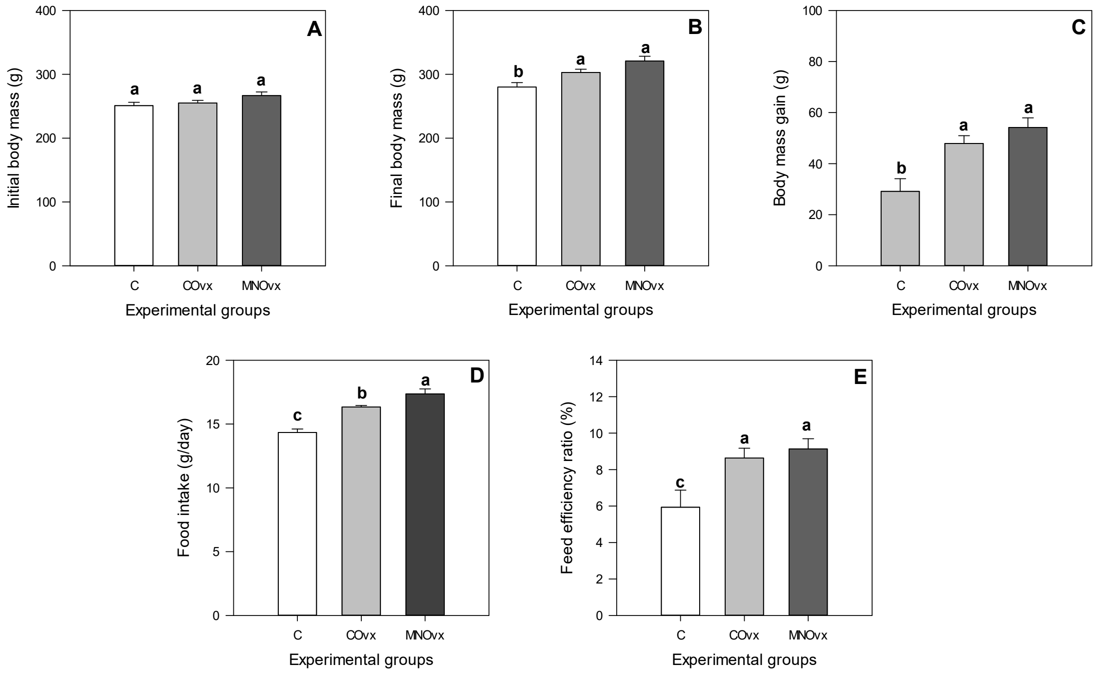
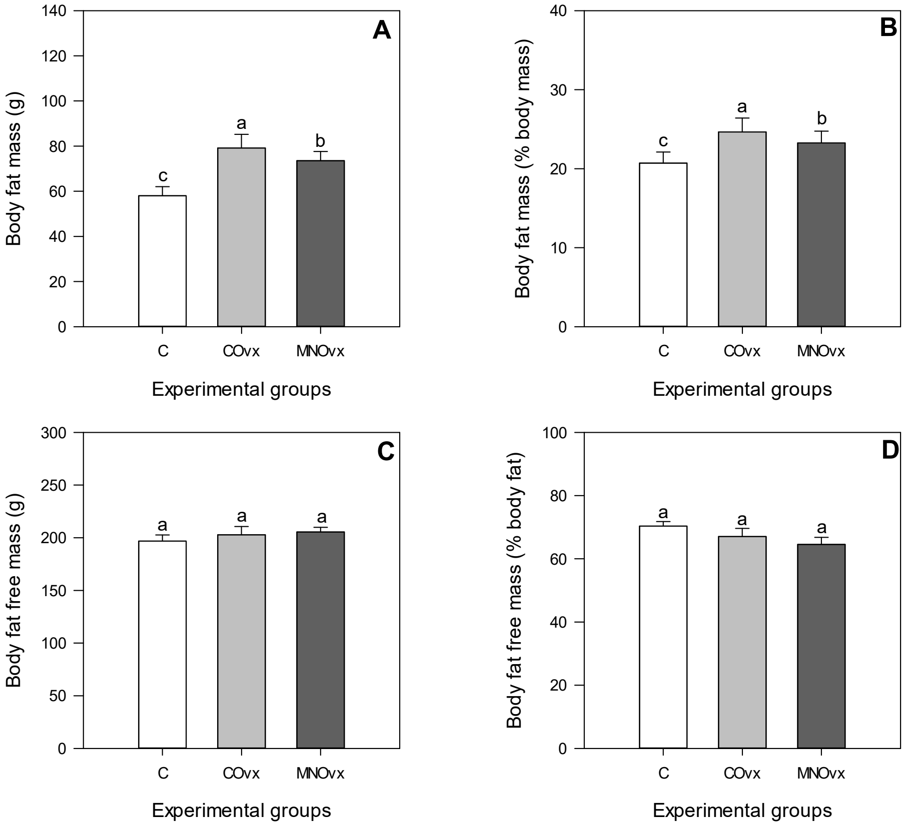
Figure 1 – Effects of the hydroalcoholic blackberry leaf extract on body mass, food intake, and food efficiency ratio of ovariectomized rats.



Experimental groups: C = control group, not ovariectomized; COvx: control group, ovariectomized; MNOvx: ovariectomized group treated with hydroalcoholic extract of *M nigra* leaves (400mg.kg^{-1} of body mass). Bars express mean ±standard deviation. Bars followed by different letters differ by One-way ANOVA and Tukey test ($p<0.05$). Source: Authors.

The MNOvx group gained less fat mass (74.25 ±15.40) than the COvx (76.0 ±20.68, $p<0.05$; Figure 2A and B), and C was the leanest (58.0 ±13.98, $p<0.05$; Figure 2A and B). There was no significant difference between the experimental groups for fat-free mass (C= 196.80 ±20.31; COvx= 202.80 ±27.19; MNOvx= 205.50±15.04; Figure 2C and D).

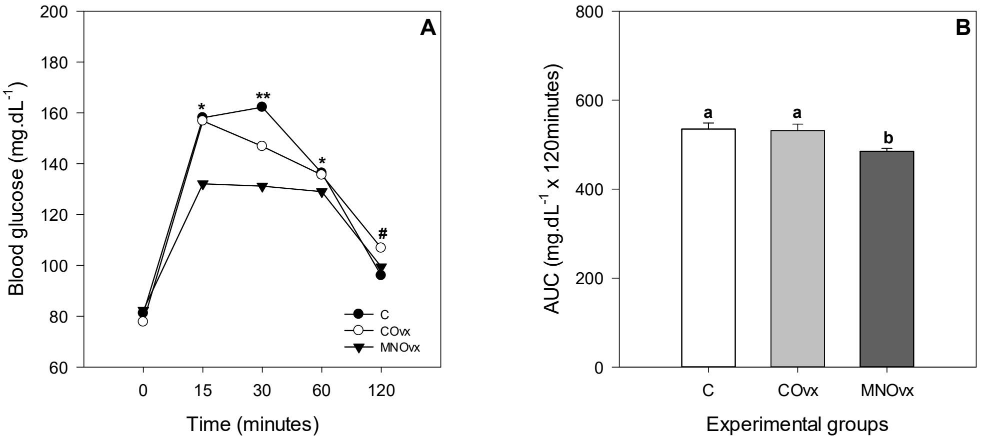
Figure 2 – Effects of the hydroalcoholic blackberry leaf extract on the body composition of ovariectomized rats.



Body fat mass (A) and body fat mass as a percentage of body mass (B); body lean mass (C) and body lean mass as a percentage of body mass (D). Experimental groups: C = control group, not ovariectomized; COvx: control group, ovariectomized; MNOvx: ovariectomized group treated with hydroalcoholic extract of *M nigra* leaves (400mg.kg^{-1} of body mass). Bars express mean ±standard deviation. Bars followed by different letters differ by One-way ANOVA and Tukey test ($p<0.05$). Source: Authors.

In the oral glucose tolerance test, the MNOvx group maintained glycemia lower than the C and COvx groups from 15 to 60 minutes ($p<0.05$, Figure 3A). At 120 minutes, the C and MNOvx groups had equally lower glycemia than the COvx group ($p<0.05$; Figure 3A). Animals in the MNOvx group were more tolerant to glucose than the C and COvx groups ($p<0.05$, Figure 3B).

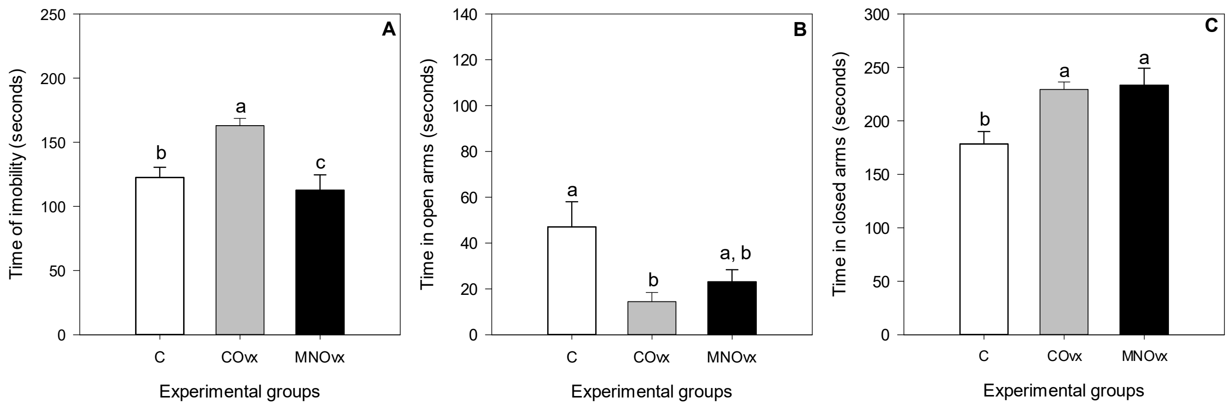
Figure 3 - Effects of hydroalcoholic blackberry leaf extract on glucose tolerance of ovariectomized rats.



Blood glucose concentration over time in the oral glucose tolerance test (A). AUC = Area under the curve (B). Experimental groups: C = control group, not ovariectomized; COvx: control group, ovariectomized; MNOvx: ovariectomized group treated with hydroalcoholic extract of *M nigra* leaves (400mg.kg^{-1} of body mass). Bars express mean ±standard deviation. Bars followed by different letters differ by One-way ANOVA and Tukey test (p<0.05). Source: Authors.

In the forced swimming test, the immobility time was lower for the MNOvx (104,74 $\pm$ 32,31) group, followed by C (127,62 $\pm$ 28,11) and, at last, COvx (162,97 $\pm$ 17,74) (Figure 4A; p<0.05). In the elevated plus maze test, COvx and (14,47 $\pm$ 12,44) spent less time in the open arms compared to C (47,09 $\pm$ 34,64); the MNOvx group (23,10 $\pm$ 16,69) did not differ from the C or COvx (Figure 4B). In the closed arms, the MNOvx and COvx groups spent more time than C (Figure 4C).

Figure 4 - Effects of hydroalcoholic blackberry leaf extract on anxiety and depression-like behaviors of ovariectomized rats.



(A) Time of immobility in the forced swimming test; Time in open (B) and closed (C) arms in the elevated plus maze test Experimental groups: C = control group, not ovariectomized; COvx: control group, ovariectomized; MNOvx: ovariectomized group treated with hydroalcoholic extract of *M nigra* leaves (400mg.kg^{-1} of body mass). Bars express mean ±standard deviation. Bars followed by different letters differ by One-way ANOVA and Tukey test (p<0.05). Source: Authors.

4. Discussion

This study investigated the effects of a hydroalcoholic extract of *M. nigra* leaves (MN) on body composition, glucose tolerance, anxiety, and depression-like behaviors in ovariectomized rats as an experimental model of menopause. In general, the MN increased food intake without significantly increasing fat mass. It also improved glucose tolerance in the ovariectomized rats. In addition, depression-like behavior was also attenuated by the MN.

It has been shown that an increase in body fat results from energy imbalance during the menopause transition, and several factors could be associated. For example, women in this period tend to be less physically active. Also, it seems there is a reduction in their metabolic rate (Lovejoy et al., 2008). Hormonal changes significantly impact food intake. Physiologically, estrogens send messages to the brain contributing to reducing appetite and increasing satiety. In the menopause transition, the decline in the production of these hormones leads to a greater food intake, especially of sugar or other refined carbohydrate-rich foods, which favors body fat gain (Espeland et al., 1997). Indeed, in our study, the ovariectomy led to a higher food intake and body mass, and this effect was even more prominent in MNL rats.

Conversely, the MNOvx rats were less efficient in gaining body or fat mass. We could not evaluate adipocyte histomorphometry in this study, but we believe that they reduced in size in response to a lower deposition of triacylglycerols (TAG), especially in visceral adipocytes. In this sense, MN may have contributed to the reduction of lipolysis in adipose tissue, thus reducing TAG deposition. In an animal model of pigs (cardiovascular system like ours), Fan et al. (2020) showed that a diet supplemented with 5% *M. nigra* leaf powder increased the hormone-sensitive lipase and inhibited the fatty acid synthase synthesis from adipose tissue. These effects could have increased the lipolysis of subcutaneous adipose tissue, which was reflected in a lower fat mass. Therefore, in our study, we can speculate that MN protected ovariectomized rats from fat mass accumulation.

Impaired glucose tolerance is one diagnostic criterion of prediabetes, which puts people at high risk of developing type II diabetes (Carracher et al., 2018; IDF Diabetes Atlas - 8th edition, 2017). Changes in reproductive hormones and body composition across the menopause transition are associated with impaired glucose homeostasis. A role for estrogens (mainly E2) in maintaining glucose homeostasis through effects on insulin secretion and clearance has been suggested. In postmenopausal women without obesity, an increase in abdominal fat during the menopause transition was associated with decreased tissue insulin sensitivity and glucose tolerance (Godsland, 2005). The increased abdominal adiposity observed during the menopause transition is likely to be associated with insulin resistance and reduced glucose tolerance (Marlatt et al., 2022). Indeed, it is widely described that the accumulation of TAG in adipose tissue is associated with the development of glucose metabolism disorders, such as low glucose tolerance and insulin resistance. TAG accumulation in adipose tissue increases the production and release of free fatty acids (FFA), reactive oxygen species (ROS), and pro-inflammatory cytokines into the circulation. Excess FFA enters non-fat cells of organs such as the liver, muscle, and pancreas and remains ectopic fat, causing lipotoxicity. Toxic lipids disrupt cellular organelles, such as mitochondria and endoplasmic reticulum, producing and releasing excess ROS and pro-inflammatory cytokines. This process disrupts the insulin signaling pathway and glucose homeostasis.

Conversely, reducing body fat reverses or attenuates this process, contributing to the re-establishment of homeostasis (Ahmed et al., 2021). In our study, despite the greater body mass, there was a lower fat mass in the ovariectomized rats that received MN; therefore, this may have contributed to the improvement in glucose tolerance observed in these animals. The hypoglycemic effect of *M. nigra* hydroalcoholic extracts has been demonstrated in animal models. In the study by Abouzid et al. (2014) an 80% hydroalcoholic extract significantly decreased fasting glycemia and increased serum insulin in streptozotocin-treated rats. In the Araujo et al. (2015) study, alloxan-treated rats receiving a 70% hydroalcoholic extract improved the systemic redox state and insulin sensitivity and reduced circulating glucose. In another study, a 90% extract reduced fasting and postprandial blood glucose and improved glucose tolerance (Júnior et al., 2017). Such effects have been attributed to the biological properties of several bioactive compounds from *M. nigra* leaves, most of all antioxidants, especially β-sitosterol, kaempferol 3-O-glycoside, and quercetin 3-O-glycoside (Moura et al., 2019). β-sitosterol has been shown to enhance glycemic control by increasing insulin receptor and glucose transporter 4 (GLUT4) activation in various tissues, such as adipose (Ponnulakshmi et al., 2019). Kaempferol can protect pancreatic cells (Alkhalidy et al., 2015) and inhibit apoptosis, promote proliferation, and increase the number of β-pancreatic islets (Zhang et al., 2013) and improve insulin sensitivity by increasing the expression of phosphorylated AMPK. Quercetin, in turn, can improve insulin sensitivity and reduce blood glucose because it reduces endoplasmic reticulum stress and improves pancreatic redox state, in addition to reducing beta cell apoptosis (Gaballah et al., 2017). Therefore, these compounds, together, may have been responsible, at least in part, for the restoration of glucose tolerance in ovariectomized rats MN-treated. Additional studies are needed to try to elucidate mechanisms that support these findings.

Cognitive and mood changes are frequently mentioned as complaints before, during, and after the menopausal transition. There is substantial biological evidence for such associations to occur, as there are many mechanisms through which estrogens can affect the brain, and its role in regulating metabolism is one of them (Hogervorst et al., 2022). In this sense, it has been shown that glucose homeostasis impairments could be associated with these disturbances. Alterations in insulin levels might affect neuronal glucose uptake and metabolism via the translocation of glucose transporter 4 (GLUT4) in response to insulin/IRS-1/Akt signaling in the brain regions, which is essential for cognitive and emotional function (Arnold et al., 2018). Insulin receptor substrate-1 (IRS-1) activates phosphoinositide 3-kinases (PI3K) via an interaction with its regulatory subunit, resulting in the subsequent phosphorylation of protein kinase B (Akt), which is a central intermediate for many of the metabolic actions of insulin (Xu et al., 2018). The PI3K/Akt activation increases glucose uptake by stimulating the movement of the GLUT4 isoform to the plasma membrane (Saltiel & Kahn, 2001). Our study could not measure insulin resistance, but ovariectomized MN-treated rats improved glucose tolerance and depression-like behavior. Some also have shown the effects of blackberry leaf extracts on depression-like behavior. Dalmagro et al. (2017) also showed a reduction in immobility time in the swimming forced test in mice treated with an aqueous extract of *M. nigra* (3-100 $mg.kg^{-1}$). Later, they attributed this effect to a nitro-oxidative modulation in the serum and the brain and protection against cell death induced by glutamate. They speculated that, at least in part, these effects were due to syringic acid, one of the main phenolics in the *M. nigra* leaves (Dalmagro et al., 2019).

Regarding anxiety-like behaviors, we did not observe the effects of MN in the ovariectomized rats. Fernández-Demeneghi et al. (2019) tested a blackberry juice (fruit) in the anxiety-like behavior (elevated plus maze test) of rats. They observed no effects at low (2.6 mg/kg anthocyanins, 14.57 mg/kg polyphenols) and high doses (10.57 mg/kg anthocyanins, 38.40 mg/kg polyphenols), but intermediate doses (5.83 mg/kg anthocyanins, 27.10 mg/kg polyphenols) decreased anxiety-like behaviors. To our knowledge, few studies are consistent regarding to effects of *M. nigra* extracts (leaves or fruits) on anxiety-like behavior.

5. Conclusion

The treatment with MN protected the ovariectomized rats from gaining body fat, which may have contributed to maintaining their glucose tolerance and, finally, to improving depression-like behavior. It is possible that antioxidant compounds from *M. nigra* leaves, mainly phenolic, may be involved in these effects. Further investigations must be carried out in order to find a minimum dose by which the beneficial effects could be observed, as well as to identify and elucidate mechanisms involved in these effects.

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References

Abouzid, S. F., Ahmed, O. M., Ahmed, R. R., Mahmoud, A., Abdella, E., & Ashour, M. B. (2014). Antihyperglycemic effect of crude extracts of some Egyptian plants and algae. *Journal of Medicinal Food*, *17*(3), 400–406. https://doi.org/10.1089/JMF.2013.0068

Ahmed, B., Sultana, R., & Greene, M. W. (2021). Adipose tissue and insulin resistance in obese. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *137*. https://doi.org/10.1016/J.BIOPHA.2021.111315

Alkhalidy, H., Moore, W., Zhang, Y., McMillan, R., Wang, A., Ali, M., Suh, K. S., Zhen, W., Cheng, Z., Jia, Z., Hulver, M., & Liu, D. (2015). Small Molecule Kaempferol Promotes Insulin Sensitivity and Preserved Pancreatic β -Cell Mass in Middle-Aged Obese Diabetic Mice. *Journal of Diabetes Research*, *2015*. https://doi.org/10.1155/2015/532984

Al-Safi, Z. A., & Polotsky, A. J. (2015). Obesity and menopause. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *29*(4), 548–553. https://doi.org/10.1016/J.BPOBGYN.2014.12.002

ANVISA. (2007). *RESOLUÇÃO-RDC Nº 67, DE 8 DE OUTUBRO DE 2007*. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2007/res0067_08_10_2007.html

Araujo, C. M., Lúcio, K. D. P., Silva, M. E., Isoldi, M. C., de Souza, G. H. B., Brandão, G. C., Schulz, R., & Costa, D. C. (2015). Morus nigra leaf extract improves glycemic response and redox profile in the liver of diabetic rats. *Food & Function*, *6*(11), 3490–3499. https://doi.org/10.1039/C5FO00474H

Arnold, S. E., Arvanitakis, Z., Macauley-Rambach, S. L., Koenig, A. M., Wang, H. Y., Ahima, R. S., Craft, S., Gandy, S., Buettner, C., Stoeckel, L. E., Holtzman, D. M., & Nathan, D. M. (2018). Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nature Reviews. Neurology*, *14*(3), 168–181. https://doi.org/10.1038/NRNEUROL.2017.185

Bruns, C. M., & Kemnitz, J. W. (2004). Sex hormones, insulin sensitivity, and diabetes mellitus. *ILAR Journal*, *45*(2), 160–169. https://doi.org/10.1093/ILAR.45.2.160

Carracher, A. M., Marathe, P. H., & Close, K. L. (2018). International Diabetes Federation 2017. *Journal of Diabetes*, *10*(5), 353–356. https://doi.org/10.1111/1753-0407.12644

Dalmagro, A. P., Camargo, A., Severo Rodrigues, A. L., & Zeni, A. L. B. (2019). Involvement of PI3K/Akt/GSK-3β signaling pathway in the antidepressant-like and neuroprotective effects of Morus nigra and its major phenolic, syringic acid. *Chemico-Biological Interactions*, *314*. https://doi.org/10.1016/J.CBI.2019.108843

Dalmagro, A. P., Camargo, A., & Zeni, A. L. B. (2017). Morus nigra and its major phenolic, syringic acid, have antidepressant-like and neuroprotective effects in mice. *Metabolic Brain Disease*, *32*(6), 1963–1973. https://doi.org/10.1007/S11011-017-0089-Y

de Paoli, M., Zakharia, A., & Werstuck, G. H. (2021). The Role of Estrogen in Insulin Resistance: A Review of Clinical and Preclinical Data. *The American Journal of Pathology*, *191*(9), 1490–1498. https://doi.org/10.1016/J.AJPATH.2021.05.011

Edwards, B. J., & Li, J. (2013). Endocrinology of menopause. *Periodontology 2000*, *61*(1), 177–194. https://doi.org/10.1111/J.1600-0757.2011.00407.X

Espeland, M. A., Stefanick, M. L., Kritz-Silverstein, D., Fineberg, S. E., Waclawiw, M. A., James, M. K., & Greendale, G. A. (1997). Effect of postmenopausal hormone therapy on body weight and waist and hip girths. *Journal of Clinical Endocrinology and Metabolism*, *82*(5), 1549–1556. https://doi.org/10.1210/JC.82.5.1549

Fan, L., Peng, Y., Wu, D., Hu, J., Shi, X., Yang, G., & Li, X. (2020). Dietary supplementation of Morus nigra L. leaves decrease fat mass partially through elevating leptin-stimulated lipolysis in pig model. *Journal of Ethnopharmacology*, *249*. https://doi.org/10.1016/J.JEP.2019.112416

Fernández-Demeneghi, R., Rodríguez-Landa, J. F., Guzmán-Gerónimo, R. I., Acosta-Mesa, H. G., Meza-Alvarado, E., Vargas-Moreno, I., & Herrera-Meza, S. (2019). Effect of blackberry juice (Rubus fruticosus L.) on anxiety-like behaviour in Wistar rats. *International Journal of Food Sciences and Nutrition*, *70*(7), 856–867. https://doi.org/10.1080/09637486.2019.1580680

Figueiredo, C. P., Barros-Aragão, F. G. Q., Neris, R. L. S., Frost, P. S., Soares, C., Souza, I. N. O., Zeidler, J. D., Zamberlan, D. C., de Sousa, V. L., Souza, A. S., Guimarães, A. L. A., Bellio, M., Marcondes de Souza, J., Alves-Leon, S. v., Neves, G. A., Paula-Neto, H. A., Castro, N. G., de Felice, F. G., Assunção-Miranda, I., & Ferreira, S. T. (2019). Zika virus replicates in adult human brain tissue and impairs synapses and memory in mice. *Nature Communications 2019 10:1*, *10*(1), 1–16. https://doi.org/10.1038/s41467-019-11866-7

Fraulob, J. C., Ogg-Diamantino, R., Fernandes-Santos, C., Aguila, M. B., & Mandarim-de-Lacerda, C. A. (2010). A Mouse Model of Metabolic Syndrome: Insulin Resistance, Fatty Liver and Non-Alcoholic Fatty Pancreas Disease (NAFPD) in C57BL/6 Mice Fed a High Fat Diet. *Journal of Clinical Biochemistry and Nutrition*, *46*(3), 212–223. https://doi.org/10.3164/JCBN.09-83

Gaballah, H. H., Zakaria, S. S., Mwafy, S. E., Tahoon, N. M., & Ebeid, A. M. (2017). Mechanistic insights into the effects of quercetin and/or GLP-1 analogue liraglutide on high-fat diet/streptozotocin-induced type 2 diabetes in rats. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *92*, 331–339. https://doi.org/10.1016/J.BIOPHA.2017.05.086

Godsland, I. F. (2005). Oestrogens and insulin secretion. *Diabetologia*, *48*(11), 2213–2220. https://doi.org/10.1007/S00125-005-1930-0

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., Sherman, S., Sluss, P. M., & de Villiers, T. J. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *The Journal of Clinical Endocrinology and Metabolism*, *97*(4), 1159–1168. https://doi.org/10.1210/JC.2011-3362

Hogervorst, E., Craig, J., & O'Donnell, E. (2022). Cognition and mental health in menopause: A review. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *81*, 69–84. https://doi.org/10.1016/J.BPOBGYN.2021.10.009

IDF Diabetes Atlas – (8th edition). (2017). *8th edition - IDF Diabetes Atlas*. https://diabetesatlas.org/atlas/eighth-edition/

Jaballah, A., Soltani, I., Bahia, W., Dandana, A., Hasni, Y., Miled, A., & Ferchichi, S. (2021). The Relationship Between Menopause and Metabolic Syndrome: Experimental and Bioinformatics Analysis. *Biochemical Genetics*, *59*(6), 1558–1581. https://doi.org/10.1007/S10528-021-10066-7

Johnson, A., Roberts, L., & Elkins, G. (2019). Complementary and Alternative Medicine for Menopause. *Journal of Evidence-Based Integrative Medicine*, *24*, 1–14. https://doi.org/10.1177/2515690X19829380

Jones, G. L., & Sutton, A. (2008). Quality of life in obese postmenopausal women. *Menopause International*, *14*(1), 26–32. https://doi.org/10.1258/MI.2007.007034

Júnior, I. I. D. S., Barbosa, H. D. M., Carvalho, D. C. R., Barros, R. D. A., Albuquerque, F. P., da Silva, Di. H. A., Souza, G. R., Souza, N. A. C., Rolim, L. A., Silva, F. M. M., Duarte, G. I. B. P., Almeida, J. R. G. D. S., de Oliveira Júnior, F. M., Gomes, D. A., & Lira, E. C. (2017). Brazilian Morus nigra Attenuated Hyperglycemia, Dyslipidemia, and Prooxidant Status in Alloxan-Induced Diabetic Rats. *TheScientificWorldJournal*, *2017*. https://doi.org/10.1155/2017/5275813

Lim, S. H., & Choi, C. I. (2019). Pharmacological Properties of Morus nigra L. (Black Mulberry) as A Promising Nutraceutical Resource. *Nutrients*, *11*(2). https://doi.org/10.3390/NU11020437

Lizcano, F., & Guzmán, G. (2014). Estrogen Deficiency and the Origin of Obesity during Menopause. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/757461

Lock, M. (1991). Contested meanings of the menopause. *Lancet (London, England)*, *337*(8752), 1270–1272. https://doi.org/10.1016/0140-6736(91)92931-Q

Lovejoy, J. C., Champagne, C. M., de Jonge, L., Xie, H., & Smith, S. R. (2008). Increased visceral fat and decreased energy expenditure during the menopausal transition. *International Journal of Obesity (2005)*, *32*(6), 949–958. https://doi.org/10.1038/IJO.2008.25

Lund, K. S., Siersma, V. D., Christensen, K. B., Waldorff, F. B., & Brodersen, J. (2018). Measuring bothersome menopausal symptoms: Development and validation of the MenoScores questionnaire. *Health and Quality of Life Outcomes*, *16*(1), 1–12. https://doi.org/10.1186/S12955-018-0927-6/TABLES/5

Marlatt, K. L., Pitynski-Miller, D. R., Gavin, K. M., Moreau, K. L., Melanson, E. L., Santoro, N., & Kohrt, W. M. (2022). Body composition and cardiometabolic health across the menopause transition. *Obesity (Silver Spring, Md.)*, *30*(1), 14–27. https://doi.org/10.1002/OBY.23289

Marlatt, K. L., Redman, L. M., Beyl, R. A., Smith, S. R., Champagne, C. M., Yi, F., & Lovejoy, J. C. (2020). Racial differences in body composition and cardiometabolic risk during the menopause transition: a prospective, observational cohort study. *American Journal of Obstetrics and Gynecology*, *222*(4), 365.e1-365.e18. https://doi.org/10.1016/J.AJOG.2019.09.051

Monteleone, P., Mascagni, G., Giannini, A., Genazzani, A. R., & Simoncini, T. (2018). Symptoms of menopause - global prevalence, physiology and implications. *Nature Reviews. Endocrinology*, *14*(4), 199–215. https://doi.org/10.1038/NRENDO.2017.180

Moreira, M. de A., Brito, M. V. H., Brito, N. M. B., & Freire Filho, M. de S. L. (2005). Estudo histomorfométrico do músculo esquelético de ratos em anestro. *Acta Cirúrgica Brasileira*, *20*(4), 329–335. https://doi.org/10.1590/S0102-86502005000400012

Moura, C. A., Oliveira-Júnior, R. G., Oliveira, A. P., Silva, A. L. N., Silva, J. M. S., Santos, R. F., Santos, M. C. M., Alves, C. S. C., Dutra, L. M., Costa, E. v, & Almeida, J. R. G. S. (2019). Chemical Constituents from the Leaves of Morus nigra L. (Moraceae) Collected in Casa Nova, Bahia, Brazil. *Revista Virtual de Química*, *11*(2), 394–400. https://doi.org/10.21577/1984-6835.20190029

Obermeyer, C. M. (2000). Menopause across cultures: a review of the evidence. *Menopause (New York, N.Y.)*, *7*(3), 184–192. https://doi.org/10.1097/00042192-200007030-00009

Palacios, S., Stevenson, J. C., Schaudig, K., Lukasiewicz, M., & Graziottin, A. (2019). Hormone therapy for first-line management of menopausal symptoms: Practical recommendations. *Women's Health*, *15*. https://doi.org/10.1177/1745506519864009

Polumackanycz, M., Wesolowski, M., & Viapiana, A. (2021). Morus alba L. and Morus nigra L. Leaves as a Promising Food Source of Phenolic Compounds with Antioxidant Activity. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *76*(4), 458–465. https://doi.org/10.1007/S11130-021-00922-7

Ponnulakshmi, R., Shyamaladevi, B., Vijayalakshmi, P., & Selvaraj, J. (2019). In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. *Toxicology Mechanisms and Methods*, *29*(4), 276–290. https://doi.org/10.1080/15376516.2018.1545815

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., Jackson, R. D., Beresford, S. A. A., Howard, B. v., Johnson, K. C., Kotchen, J. M., & Ockene, J. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*, *288*(3), 321–333. https://doi.org/10.1001/JAMA.288.3.321

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, *414*(6865), 799–806. https://doi.org/10.1038/414799A

Savergnini, S. Q., Reis, A. M., Santos, R. A. S., Santos, P. E. B., Ferreira, A. J., & Almeida, A. P. (2012). Effects of short-term administration of estradiol on reperfusion arrhythmias in rats of different ages. *Brazilian Journal of Medical and Biological Research*, *45*(12), 1248–1254. https://doi.org/10.1590/S0100-879X2012007500169

Shifren, J. L., Gass, M. L. S., Kagan, R., Kaunitz, A. M., Liu, J. H., Pinkerton, J. A. v., Schnatz, P. F., Stuenkel, C. A., Allam, S. H., Allen, R. H., Bachmann, G. A., Merz, C. N. B., Bergfeld, W. F., Block, J. A., Clarkson, T. B., Clayton, J. A., Cwiak, C., Davis, S. R., Diab, D., & Schiff, I. (2014). The North American Menopause Society recommendations for clinical care of midlife women. *Menopause (New York, N.Y.)*, *21*(10), 1038–1062. https://doi.org/10.1097/GME.0000000000000319

Sinatora, R. V., Chagas, E. F. B., Mattera, F. O. P., Mellem, L. J., dos Santos, A. R. de O., Pereira, L. P., Aranão, A. L. de C., Guiguer, E. L., Araújo, A. C., Haber, J. F. D. S., Guissoni, L. C., & Barbalho, S. M. (2022). Relationship of Inflammatory Markers and Metabolic Syndrome in Postmenopausal Women. *Metabolites*, *12*(1). https://doi.org/10.3390/METABO12010073

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols 2012 7:6*, *7*(6), 1009–1014. https://doi.org/10.1038/nprot.2012.044

Soares, C. N. (2007). Menopausal transition and depression: who is at risk and how to treat it? *Expert Review of Neurotherapeutics*, *7*(10), 1285–1293. https://doi.org/10.1586/14737175.7.10.1285

Sokri, M. A., Hussin, M. M., Pahirulzaman, K. A. K., & Saidan, N. H. (2021). Effectiveness of ethanolic and methanolic Morus nigra extracts on microbial strains. *AIP Conference Proceedings*, *2347*(1), 020153. https://doi.org/10.1063/5.0051514

Thurston, R. C., Sowers, M. F. R., Sutton-Tyrrell, K., Everson-Rose, S. A., Lewis, T. T., Edmundowicz, D., & Matthews, K. A. (2008). Abdominal adiposity and hot flashes among midlife women. *Menopause (New York, N.Y.)*, *15*(3), 429–434. https://doi.org/10.1097/GME.0B013E31815879CF

Thurston, R. C., Sowers, M. R., Sternfeld, B., Gold, E. B., Bromberger, J., Chang, Y., Joffe, H., Crandall, C. J., Waetjen, L. E., & Matthews, K. A. (2009). Gains in body fat and vasomotor symptom reporting over the menopausal transition: the study of women's health across the nation. *American Journal of Epidemiology*, *170*(6), 766–774. https://doi.org/10.1093/AJE/KWP203

Voedisch, A. J., Dunsmoor-Su, R., & Kasirsky, J. (2021). Menopause: A Global Perspective and Clinical Guide for Practice. *Clinical Obstetrics and Gynecology*, *64*(3), 528–554. https://doi.org/10.1097/GRF.0000000000000639

Wadden, T. A., Butryn, M. L., Sarwer, D. B., Fabricatore, A. N., Crerand, C. E., Lipschutz, P. E., Faulconbridge, L., Raper, S., & Williams, N. N. (2006). Comparison of psychosocial status in treatment-seeking women with class III vs. class I–II obesity. *Surgery for Obesity and Related Diseases*, *2*(2), 138–145. https://doi.org/10.1016/J.SOARD.2006.03.016

Wang, L., Wang, J., Ma, M., Shen, L., Huang, T., Huang, C., Jia, A., & Hu, X. (2022). Prenylated flavonoids from Morus nigra and their insulin sensitizing activity. *Phytochemistry*, *203*, 113398. https://doi.org/10.1016/J.PHYTOCHEM.2022.113398

Xu, Y., Fu, J. F., Chen, J. H., Zhang, Z. W., Zou, Z. Q., Han, L. Y., Hua, Q. H., Zhao, J. S., Zhang, X. H., & Shan, Y. J. (2018). Sulforaphane ameliorates glucose intolerance in obese mice via the upregulation of the insulin signaling pathway. *Food & Function*, *9*(9), 4695–4701. https://doi.org/10.1039/C8FO00763B

Yan, H., Yang, W., Zhou, F., Li, X., Pan, Q., Shen, Z., Han, G., Newell-Fugate, A., Tian, Y., Majeti, R., Liu, W., Xu, Y., Wu, C., Allred, K., Allred, C., Sun, Y., & Guo, S. (2019). Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1. *Diabetes*, *68*(2), 291–304. https://doi.org/10.2337/DB18-0638

Zhang, Y., Zhen, W., Maechler, P., & Liu, D. (2013). Small molecule kaempferol modulates PDX-1 protein expression and subsequently promotes pancreatic β-cell survival and function via CREB. *The Journal of Nutritional Biochemistry*, *24*(4), 638–646. https://doi.org/10.1016/J.JNUTBIO.2012.03.008