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Betulinic acid and obesity-related disorders

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The obesity epidemic is not just a health issue, it is increasingly driving a shift in the prevalence of chronic diseases, affecting 890 million adults and straining healthcare systems worldwide. Conditions such as type 2 diabetes mellitus, cardiovascular diseases, non-alcoholic fatty liver disease, and various cancer types are closely tied to this growing crisis. Betulinic acid has anti-inflammatory, antioxidant and anti-cancer properties and modulates key metabolic pathways such as NF- κ B and AMPK signaling. This compound improves insulin sensitivity, reduces hepatic steatosis, mitigates the progression of atherosclerosis and fibrosis, and suppresses inflammatory responses, which are important in treating those obesity-related disorders. Additionally, betulinic acid use in cancer treatment has been explored due to its potential in angiogenesis and metastasis inhibition and promotion of apoptosis. This review spotlights the therapeutic potential of the natural compound betulinic acid in processes such as insulin sensitivity, glucose and lipid metabolism, adiposity, inflammation, oxidative stress, intestinal microbiota, and other mechanisms underlying different obesity-related disorders. Overall, besides strong therapeutic potential of betulinic acid, described limitations such as poor aqueous solubility, limited bioavailability, production and extraction have resulted in scarce clinical data making it premature to draw definitive conclusions regarding its application in clinical practice.

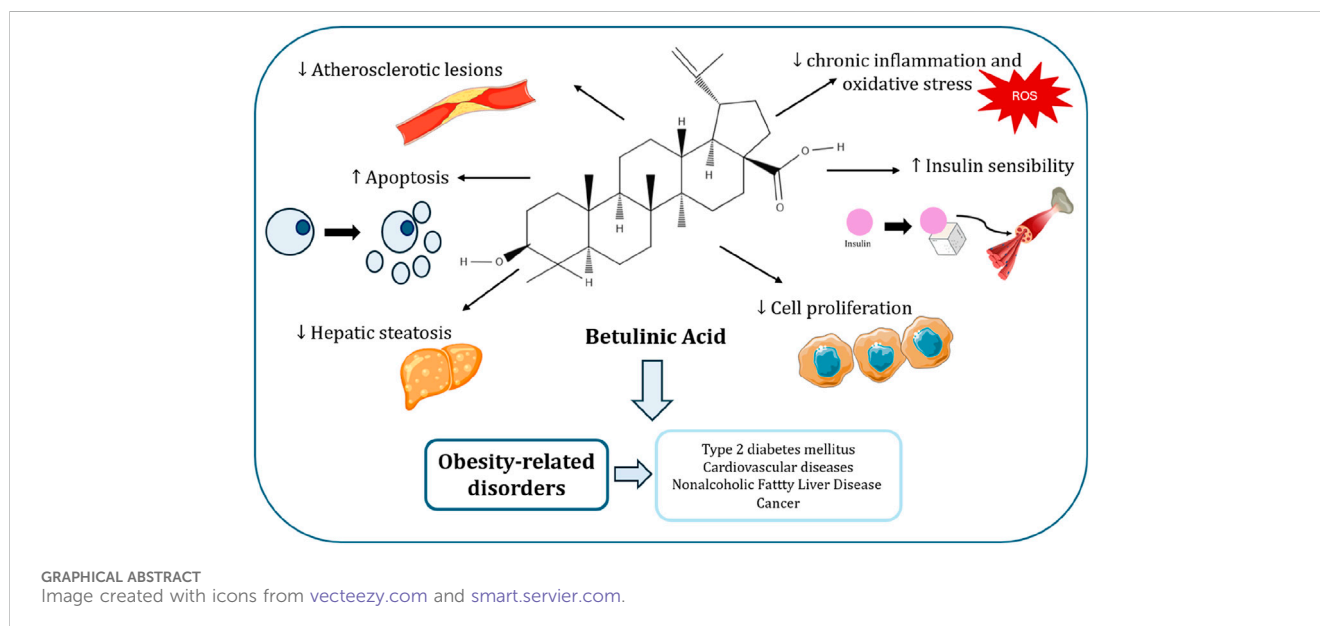
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1 Introduction

Obesity is described as an excessive accumulation of body fat and is typically diagnosed when an individual's body mass index (BMI) surpasses 30 kg/m², according to the World Health Organization (WHO) (WHO, Obesity, 2025). Globally, obesity has been on the rise, affecting 890 million adults and significantly increasing the risk of mortality, highlighting the need for global preventive measures and effective interventions (WHO, 2025; Berrington de Gonzalez et al., 2010).

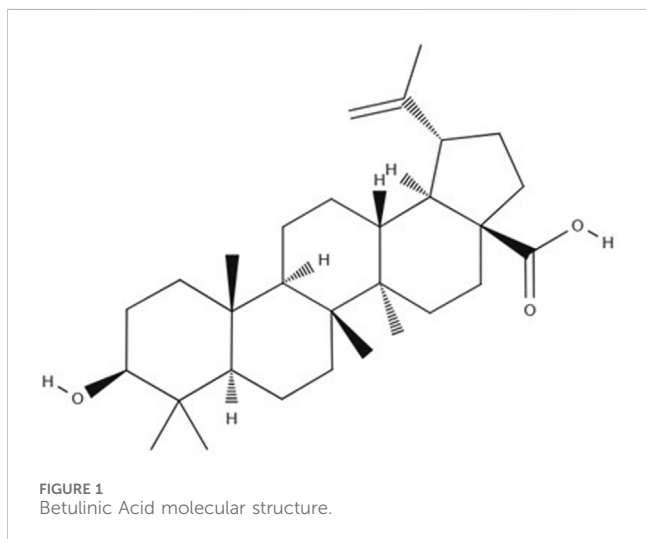
Obesity is related with multiple health issues, such as type 2 diabetes mellitus (T2DM), dyslipidemia, cardiovascular diseases (CVD), respiratory problems and many cancers, also called Obesity-Related Disorders (ORD) (Apovian, 2016). Furthermore, the interplay between genetic, environmental, and behavioural factors contributes to obesity, driven by greater food accessibility, reduced physical activity, and genetic predisposition (Singh et al., 2020). The treatment of obesity effectively involves several



strategies, including dietary modifications, medication, such as Food and Drug Administration-approved drugs, and, in cases of severe obesity, bariatric surgery (Lemstra et al., 2016; Tak and Lee, 2020; Wiechert and Holzapfel, 2021).

As obesity becomes more prevalent and effective treatments increasingly difficult to achieve, there is a growing interest in alternative approaches, including natural bioactive compounds with the potential to address this health challenge. Betulinic Acid (BA) (Figure 1), a natural compound, has been demonstrated to prevent the accumulation of abdominal fat and promote weight loss, making it a promising candidate for use as a natural treatment for obesity (Zhu et al., 2024; de Melo et al., 2009).

This review aims to summarize the several ORD, reporting the mechanisms underlying these diseases, and explore the potential effect of BA in the treatment of these conditions.



2 Obesity-related disorders

2.1 Obesity and type 2 diabetes mellitus

T2DM occurs when the body cannot produce enough insulin or use the insulin that produces effectively, resulting in high levels of blood glucose (hyperglycemia) (Federation, 2025). In fact, some studies suggest that nearly 90% of T2DM occurrences are due to obesity (Pereira and Alvarez-Leite, 2014).

Insulin is fundamental for carbohydrates, proteins, and lipids regulation as it promotes glucose absorption from the bloodstream into adipose tissue, skeletal muscle cells, and liver (Wilcox, 2005). Insulin attaches to the α -subunits of its receptor, triggering the receptor's autophosphorylation and the activation of insulin receptor substrates (IRS) (Tong et al., 2022; Zhang et al., 2020). This activates protein kinase B (PKB/Akt), which is essential for bringing glucose transporter 4 (GLUT4) to the cell membrane, enabling glucose to be absorbed into the cell (Tong et al., 2022; Zhang et al., 2020). Both obesity and T2DM are closely associated with insulin resistance, a condition where cells become less sensitive to the effects of insulin (Al-Goblan et al., 2014).

An increase in fatty acids (FA) from food intake and lipolysis of the adipose tissue leads to an elevated β -oxidation and a reduction in the use of glucose for metabolic processes, such as glucose intake and glycogen synthesis (Lois and Kumar, 2009; Shulman et al., 1990; Golay et al., 1984; Felber et al., 1987). This imbalance contributes to chronic hyperglycemia, impairing insulin sensitivity and eventually resulting in insulin resistance (Verma and Hussain, 2017). In T2DM, dysfunction in pancreatic β -cells significantly induces the progression of this disease, because these cells normally increase insulin production to compensate for rising blood glucose levels, especially in obesity, where insulin secretion is elevated and insulin clearance by the liver is reduced (Kahn et al., 2006; Kahn, 2001).

Prolonged exposure to high levels of glucose and FAs can damage β -cell function (Hauner, 2016). Chronic exposure to fatty acids leads to "glucolipototoxicity," which impairs the β -cells' ability to

release insulin in response to glucose (Poitout and Robertson, 2008). This occurs through multiple pathways, including FAs binding to G-protein-coupled receptors on the β -cell membrane, which disrupts calcium balance and ultimately affects insulin secretion (Hauner, 2016; Itoh et al., 2003). Another mechanism involves the upregulation of uncoupling protein 2 (UCP-2), which interferes with the mitochondrial function essential for generating the ATP necessary for insulin secretion (Hauner, 2016; Chan et al., 2004). Therefore, defects in pancreatic β -cells function disrupt glycemic control, reducing insulin secretion and contributing to the progression of T2DM (Weyer et al., 1999; Gastaldelli et al., 2004).

Obesity also causes low-grade chronic inflammation, that is manifested by the activation of immune cells, which produces and releases pro-inflammatory cytokines such as tumour necrotic factor- α (TNF- α) and interleukin-6 (IL-6), and plays a major role in the development of T2DM (Chen et al., 2023). Those cytokines trigger the activation of c-JUN N-terminal kinase (JNK) and nuclear factor-kappa B (NF- κ B) signalling pathways, the release of cytokines and chemokines, and the attraction of immune cells (Esser et al., 2014; Hirosumi et al., 2002). JNK and NF- κ B pathways contribute to insulin resistance by facilitating the phosphorylation of serine and threonine residues on IRS, disrupting its interaction with the insulin receptor (IR), impairing downstream insulin signalling pathways and contributing to the progression of T2DM (Wu and Ballantyne, 2020).

Currently, one of the treatments for T2DM is metformin (Marín-Peñalver et al., 2016; Inzucchi et al., 2015). This compound enhances insulin sensitivity, reduces intestinal glucose absorption, and lowers hepatic glucose production by inhibiting gluconeogenesis in the liver and promoting glycolysis (Padhi et al., 2020; Quillen et al., 1999). However, this treatment has some side effects such as diarrhea, nausea, anorexia, and a decrease in the intestinal absorption of vitamin B12 (Marín-Peñalver et al., 2016). Therefore, new therapeutic approaches may be explored to provide better results for patients and resolve the challenges associated with current treatments.

2.2 Obesity and cardiovascular diseases

Obesity is closely linked to CVD such as coronary heart disease, hypertension, heart failure (HF), and ischemic stroke (Collaboration et al., 2011; Krauss et al., 1998; Kenchaiah et al., 2002). Obese patients have a twofold higher risk of developing HF relative to those with normal weight, highlighting a significant link between obesity and CVD (Kenchaiah et al., 2002). Moreover, obesity exacerbates dyslipidemia, insulin resistance, hypertension, and systemic inflammation, which are key risk factors (Ortega et al., 2016). The increase in body mass can significantly impact the cardiovascular system, leading to elevated blood volume, stroke volume, and cardiac output (Lavie et al., 2014). These changes result in higher filling volume and pressure, causing left ventricular dilation and hypertrophy, contributing to cardiac dysfunction (Lavie et al., 2014).

Adipose tissue dysfunction is strongly associated with obesity-related CVD. Excessive intake of high-calorie foods leads to adipocyte hypertrophy, lipid accumulation, and subsequent cell necrosis or apoptosis (Nakamura et al., 2014; Sun et al., 2010).

This process recruits inflammatory cells and promotes the dysfunction of adipose tissue (Nakamura et al., 2014; Sun et al., 2010). Adipose tissue produces a range of adipokines such as cytokines (e.g., TNF- α , IL-6, IL-10), chemokines (e.g., MCP-1, CCL2, CCL5), acute-phase reactants (e.g., C-reactive protein (CRP)), damage-associated molecular pattern molecules (DAMPs), pro-inflammatory factors like leptin and anti-inflammatory factors like adiponectin (Landecho et al., 2019; Rodríguez et al., 2015). In the context of obesity, the secretion of these adipokines becomes dysregulated, establishing a link between excessive and dysfunctional adiposity and an elevated CVD risk (Landecho et al., 2019; Rodríguez et al., 2015).

Adiponectin and leptin, two key adipokines, play complex roles in the development of CVD. Low circulating adiponectin levels, commonly observed in obese patients, are associated with an increased risk of obesity-related CVD (Zhao et al., 2021). Similarly, leptin, which regulates food intake and energy balance, is elevated in obese patients and promotes low-grade inflammation and influences blood pressure by modulating sympathetic nervous activity, endothelial nitric oxide (NO) production, and vasoconstriction induced by angiotensin II (Landecho et al., 2019; Muruzáb et al., 2002; Gómez-Ambrosi et al., 2002; Rodríguez et al., 2010). However, obesity-induced leptin resistance leads to hyperleptinemia, a condition characterized by excessive leptin production, which contributes to vascular inflammation, insulin resistance, oxidative stress, and left ventricular hypertrophy, ultimately increasing CVD risk (Landecho et al., 2019; Kim et al., 2016; Wallace et al., 2001).

Obesity is also linked to elevated glucose levels and increased production of reactive oxygen species (ROS) and reactive nitrogen species by the endoplasmic reticulum (ER), which disrupts insulin secretion and sensitivity (Cercato and Fonseca, 2019; Keane et al., 2015). Insulin resistance promotes dyslipidemia, which is manifested by elevated levels of triglycerides, decreased levels of high-density lipoprotein (HDL), and the formation of small dense low-density lipoproteins (Ormazabal et al., 2018; Meshkani and Adeli, 2009; Robins et al., 2011). Dyslipidemia is also characterized by high levels of low-density lipoprotein cholesterol (LDL), which promote the formation of atherosclerotic plaques on arterial walls, contributing to atherosclerosis and cardiovascular complications (Alloubani et al., 2021; Elshourbagy et al., 2014). These interconnected mechanisms highlight the association between obesity and CVD, drawing attention to the urgent need for new therapeutic strategies to improve cardiovascular outcomes.

2.3 Obesity and nonalcoholic fatty liver disease

The hallmark of nonalcoholic fatty liver disease (NAFLD) is the presence of hepatic steatosis ($\geq 5\%$ of hepatocytes) without the evidence of other liver disease etiologies, such as viral hepatitis, the use of steatogenic medications, autoimmune hepatitis, Wilson's disease, or excessive alcohol consumption (Younossi et al., 2016; Chalasani et al., 2012). NAFLD represents a major global health issue, affecting an estimated 30% of the population, with prevalence rates reaching as high as 90% among individuals with obesity (Amini-Salehi et al., 2024; Machado et al., 2006). Importantly,

NAFLD can progress to more severe issues such as cirrhosis and hepatocellular carcinoma (Polyzos et al., 2019).

NAFLD is influenced by various factors, including genetic predispositions such as polymorphisms in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) genes, dietary components (e.g., fructose), oxidative stress, adipokines, insulin resistance and gut microbiota dysbiosis (Polyzos et al., 2016; Valenti et al., 2010; Abdelmalek et al., 2010; Shanab et al., 2011; Jarrar et al., 2008; Sanyal et al., 2001). The diagnosis of NAFLD relies on the identification of hepatic steatosis through imaging techniques, including ultrasound, CT, MRI, MRS, or ultrasound-based transient elastography (FibroScan), or histological analysis (Sarwar et al., 2018; Brunner et al., 2019). Besides, blood-based biomarkers, such as elevated levels of aspartate aminotransferase levels (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT), may support the diagnosis (Brunner et al., 2019).

In obese individuals with NAFLD, lipoprotein lipase catalyzes the hydrolysis of circulating triglycerides into FA, which are then transported into hepatocytes by FA transport proteins (Perla et al., 2017; Petersen et al., 2007). This process is followed by an upregulation of *de novo* lipogenesis in the liver, resulting in excessive lipid deposition (Perla et al., 2017; Petersen et al., 2007). To attenuate this accumulation, the liver has two primary mechanisms for lipids elimination: FA oxidation, which occurs via β -oxidation and ketogenesis, and very-low-density lipoprotein (VLDL)-mediated export of triglycerides (Willis et al., 2021). Furthermore, *de novo* lipogenesis generates the intermediate metabolite, malonyl-CoA, that inhibits FA oxidation, promoting lipid accumulation and contributing to the pathogenesis of NAFLD (Willis et al., 2021; McGarry et al., 1977).

Another hallmark of NAFLD is the accumulation of triglycerides in hepatocytes as lipid droplets (Gonçalves et al., 2014). Oxidative stress, which arises from an imbalance between the production of ROS and the antioxidant defences, damages intracellular biological macromolecules, inducing cell dysfunction (Tong et al., 2022). This promotes the peroxidation of lipid and generates reactive aldehydes, amplifying hepatic inflammation by secretion of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 (Delli Bovi et al., 2021; Day, 2002). In addition to mitochondrial dysfunction, ER stress further exacerbates ROS production, activates apoptotic pathways, and heightens lipotoxicity, contributing to chronic inflammation, liver fibrosis, and the progression of NAFLD to non-alcoholic steatohepatitis (NASH) (Delli Bovi et al., 2021).

Inflammation is a key factor in the transition of NAFLD into NASH (Du Plessis et al., 2015). This transition is driven by intrahepatic factors such as oxidative stress, mitochondrial dysfunction, and ER stress, leading to injury and hepatocellular metabolic dysfunction (Peiseler et al., 2022; Hardy et al., 2016). NAFLD inflammation is also regulated by extrahepatic factors such as gut-liver axis, adipose tissue inflammation, skeletal muscle, and bone marrow precursors (Peiseler et al., 2022; Nachit et al., 2021; Bäckhed et al., 2004). In obesity, adipose tissue undergoes macrophage activation, which promotes the production of pro-inflammatory mediators such as CRP and triggers inflammatory signalling pathways in the liver, including the secretion of TNF- α , IL-6 and IL-1 β (Cordeiro et al., 2020; Weisberg et al., 2003; Guilherme et al., 2008). These cytokines, in turn, promote

enhanced lipogenesis while suppressing FA oxidation, thereby establishing a connection between excessive lipid accumulation and an inflammatory status (Cordeiro et al., 2020; Guilherme et al., 2008; Kuzminova et al., 2016). Thus, the interaction between adipose tissue and the liver involves a network of inflammatory cells, adipokines, and cytokines, which are crucial in driving metabolic dysregulation and lipotoxicity (Cordeiro et al., 2020).

Dysbiosis of the intestinal microbiota, an imbalance frequently observed in obesity, emphasizes the role of the gut-liver axis in the NAFLD development (Porr et al., 2018; Karlsson et al., 2012). The gut-liver axis includes the intestinal epithelium, a crucial component of the gut barrier, which often exhibits dysfunction, known as “leaky gut” in individuals with NAFLD (Porr et al., 2018; Miele et al., 2009; Giorgio et al., 2014). This condition enhances gut permeability, contributing to an increase of toxins in the bloodstream, thereby promoting chronic low-grade inflammation, present in both obesity and NAFLD (Pezzino et al., 2022; Cani et al., 2009; Brun et al., 2007). Therefore, studies have demonstrated that in NAFLD patients, a correlation exists between increased gut permeability and the severity of hepatic steatosis (Cani et al., 2009; Brun et al., 2007).

Therapeutic approaches for NAFLD include the use of an anti-inflammatory agent named vitamin E, which has been found to improve the histological manifestations of NASH, although it does not significantly impact fibrosis severity (Said and Akhter, 2017). However, the development of additional therapeutic strategies remains essential to enhance outcomes for patients with NAFLD.

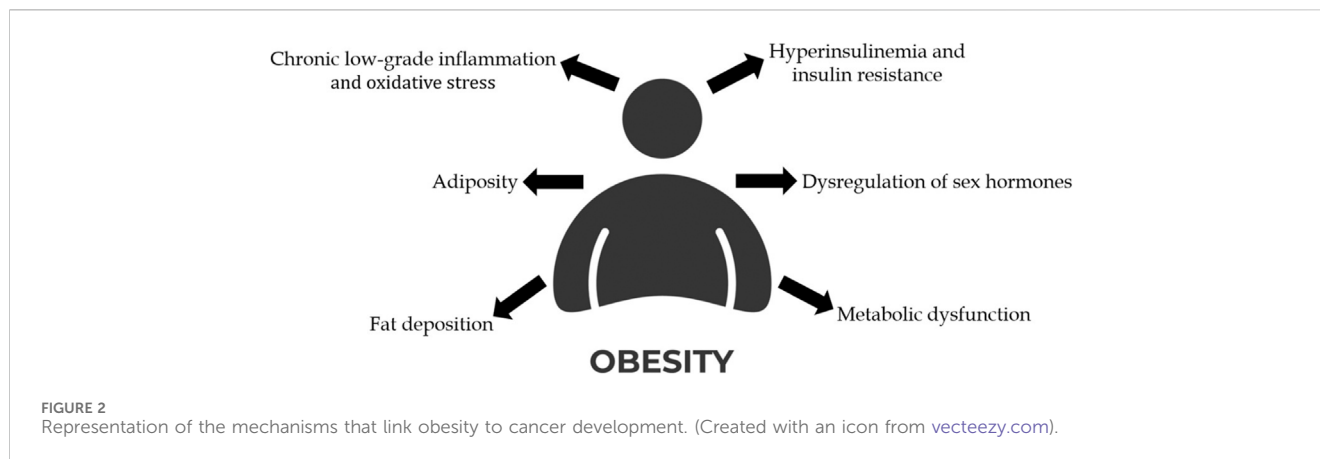
2.4 Obesity and cancer

The prevalence of cancer has been increasing over the years, with approximately 20 million new cases and 9.7 million cancer-related deaths reported worldwide in 2022 (Organization, 2024). Several risk factors have been associated with cancer, such as tobacco use, obesity, excessive alcohol consumption, a physically inactive routine and inadequate diet (Marino et al., 2024; Wu et al., 2018).

Notably, obesity is implicated in approximately 20% of all cancer cases, contributing to cancer development through several interconnected mechanisms, such as hyperinsulinemia and insulin resistance, dysregulation of sex hormones, chronic low-grade inflammation and oxidative stress, increased adiposity, the impact of ectopic fat deposition and metabolic dysfunction (Averginos et al., 2019; Karra et al., 2022; Wolin et al., 2010) (Figure 2).

Hyperinsulinemia, characterized by the increase of insulin levels due to the overproduction of insulin by pancreatic β -cells, promotes the production of insulin-like growth factor I (IGFI) (Poloz and Stambolic, 2015). This process is accompanied by reduced levels of IGF-binding proteins (IGFBP), which normally sequester IGFs in the liver (Poloz and Stambolic, 2015; Brismar et al., 1994). The resulting excess IGF and insulin activates multiple signalling pathways that promote cell proliferation, angiogenesis and inhibit apoptosis in malignant tissues (Xu et al., 2017; Tang et al., 2010; Teng et al., 2016).

Obesity induces a state of low-grade chronic inflammation primarily driven by adipose tissue, which acts as a metabolically active organ secreting adipokines and pro-inflammatory cytokines



with oncogenic potential (Booth et al., 2015). Cytokines such as IL-6, IL-1 β , and TNF- α , increase the production of CRP and serum amyloid A, both of which are implicated in facilitating tumour progression (Friedenreich et al., 2021; Zhao et al., 2010; Visser et al., 1999). Oxidative stress represents another mechanism through which obesity influences cancer progression. ROS disrupts growth factors and mitogenic pathways, thereby promoting uncontrolled cell proliferation (DeNicola et al., 2011; Lee et al., 1998).

Obesity leads to increased adiposity which is implicated in cancer progression. Adipose tissue secretes leptin and adiponectin, two hormones with opposing functions. While adiponectin levels decrease in obesity, leptin levels rise, and the resulting leptin/adiponectin ratio can be associated with enhanced cancer aggressiveness (Ryan et al., 2003; Garofalo et al., 2006; Chen et al., 2006). Furthermore, the excess of adipose tissue increases aromatase activity, resulting in elevated estrogen levels, which result in increased risk of hormone receptor-positive breast and endometrial cancers (Averginos et al., 2019; Crosbie et al., 2010). Therefore, hormonal dysregulation and increased adiposity are key mechanisms underlying the association between obesity and cancer development.

Metabolic dysfunction is another mechanism stimulated by obesity that can promote cancer progression. Warburg et al. hypothesized that dysfunction in cellular respiration serves as the root cause of all cancers, where reduced respiration triggers tumorigenesis by increasing glycolysis to compensate for the energy loss deficit (Warburg, 1956). This increased glycolytic activity leads to the production of lactate by cancer cells under aerobic conditions—a phenomenon known as aerobic glycolysis - contributing to respiratory insufficiency that may become irreversible (Seyfried et al., 2014). Although aerobic glycolysis generates less ATP per molecule of glucose compared to oxidative phosphorylation (OXPHOS), its significantly higher rate compensates by enabling greater overall energy production, which is crucial for the proliferation of cancer cells (Vander Heiden et al., 2009).

The accumulation of body fat has been identified as a contributing factor in the development of several types of cancer, including postmenopausal breast, endometrial, colorectal, esophageal adenocarcinoma and prostate cancer, among others (Leitzmann et al., 2025).

Obesity significantly increases the risk of premenopausal triple-negative breast cancer by 67% and luminal B breast cancer by 73% compared to women of normal weight, according to information from the US Cancer and Steroid Hormone Study (Turkoz et al., 2013; Gaudet et al., 2011). Several obesity-driven mechanisms, such as hypoxia, increased levels of estrogens, insulin, adipokines, inflammatory mediators, and FFA, promote cancer cell growth (Brown, 2021). Adipose tissue hosts the cytochrome P450 enzyme aromatase, which converts adrenal-produced androgen precursors into estrogens, which can contribute to tumour progression by promoting DNA damage, stimulating cell proliferation, angiogenesis, and mutagenesis (Iyengar et al., 2013; Péqueux et al., 2012; Mobley and Brueggemeier, 2004; Zhao et al., 2006). Leptin, a hormone linked to breast cancer, activates the PI3K-AKT pathway, promoting cancer cell proliferation, epithelial-mesenchymal transition (EMT), and vascular endothelial growth factor (VEGF) expression, which enhances angiogenesis and invasion (Wei et al., 2016; Gonzalez-Perez et al., 2010). Increased levels of insulin and IGF-I, which promote cell proliferation and inhibit apoptosis in breast tissue, are two ways further ways that obesity contributes to this kind of cancer (Chen et al., 2012; Weichhaus et al., 2012; Fortner et al., 2016). Additionally, obesity-induced chronic low-grade inflammation, which is characterized by enhanced macrophage infiltration and elevated expression of pro-inflammatory cytokines such as TNF- α and IL-6 in adipose tissue creates tumorigenic microenvironment (Weisberg et al., 2003).

Obesity is responsible for approximately 31% of cases of endometrial cancer, through mechanisms involving endogenous sex steroid hormones, inflammation, and insulin resistance (Pearson-Stuttard et al., 2018; Shaw et al., 2016). Notably, women with advanced-stage endometrial cancer (stage III and IV) and a BMI \geq 40 face a significantly increased mortality risk compared to those with a normal weight, showing the critical need for effective anti-obesity interventions (Modesitt et al., 2007). Type I endometrial carcinomas manifest high estrogen levels and reduced progesterone levels (Shaw et al., 2016; Key and Pike, 1988). This imbalance arises from prolonged estrogen exposure without sufficient progesterone counteraction, leading to endometrial cell proliferation and increasing the risk of malignant transformation (Shaw et al., 2016; Key and Pike, 1988). Leptin, TNF- α , and IL-6, key factors associated with obesity, promote insulin resistance and stimulate the

proliferation of endometrial cancer cells (Onstad et al., 2016). Additionally, chronic inflammation driven by obesity, enhances inflammatory cytokines levels, which promote cell proliferation and angiogenesis, consequently inducing endometrial cancer growth (Harvey et al., 2023).

Colorectal cancer is the third most prevalent cancer in the world, and its development is associated to obesity, as evidence by 2012 data showing that elevated BMI is responsible for 5.8% of new colorectal cancer cases in men and 7.0% in women (Pearson-Stuttard et al., 2018). Obesity drives inflammation through altered secretion of pro-inflammatory cytokines (e.g., L-6, TNF- α , PAI-1), adipokines, insulin, and IGF, alongside a reduction in adiponectin levels by adipose tissue (Ionescu et al., 2023). These inflammatory and hormonal changes allow the activation of signalling pathways that promote cancer cell proliferation and metastasis, thereby contributing to the development of colorectal cancer (Ionescu et al., 2023). Moreover, elevated levels of LPS, caused by gut microbiota dysbiosis, increase intestinal permeability and systemic endotoxemia, which promotes chronic inflammation that drives EMT, cell invasion, and metastasis, exacerbating colorectal cancer progression (Killeen et al., 2009; Liu et al., 2017). Additionally, bile acids secreted into the intestine induce inflammatory reactions, ROS production, epithelial damage, genomic instability, and apoptosis inhibition, collectively enhancing colorectal cancer progression (Ochsenkühn et al., 1999; Nguyen et al., 2018; Ye et al., 2020).

Esophageal adenocarcinoma (EAC) is also associated to obesity, being shown that individuals with a higher BMI face a twofold higher risk of developing EAC (Hoyo et al., 2012). Obesity causes the development of gastroesophageal reflux disease (GERD) and Barrett's esophagus, conditions that predispose individuals to carcinogenesis (Schlottmann et al., 2020). The accumulation of abdominal fat increases intra-abdominal pressure, which exerts a mechanical effect on gastric pressure, disrupting the transdiaphragmatic pressure gradient and promoting the development of GERD (El-Serag et al., 2006; De Mello Del Grande et al., 2020). A metaplastic transformation of the squamous mucosa of the esophageal epithelium in the distal esophagus with metaplastic columnar epithelium is a feature of Barrett's esophagus, which is also characterized by increased cell proliferation and reduced apoptosis (Reid et al., 1993; Jung et al., 2011). A meta-analysis demonstrated a strong association between central adiposity and an elevated risk of Barrett's esophagus, highlighting the link between obesity and this precancerous condition (Singh et al., 2013).

Prostate cancer is the second most prevalent cancer among men in the world, and obesity may accelerate its progression, because higher mortality rates are registered among those diagnosed with this disease (Sung et al., 2021; Haque et al., 2014). While a meta-analysis found no direct link between obesity and the incidence of prostate cancer, it did reveal an increased risk of aggressiveness in obese individuals (Zhang et al., 2015). Reduced prostate-specific antigen (PSA) levels in obese men may contribute to this connection by decreasing the chance of early prostate cancer detection (Vidal et al., 2014). Obesity also disrupts the insulin/IGF-1 axis, alters sex hormone levels, changes adipokine signalling, and heightens oxidative stress, all of which contribute to prostate cancer progression (Bandini et al., 2017). Hyperinsulinemia, caused by

obesity, increases IGF-1 secretion, which stimulates angiogenesis, mitogenesis, and inhibits apoptosis, processes that collectively facilitate prostate cancer progression (Bandini et al., 2017; Gallagher and LeRoith, 2010). Obesity also elevates leptin levels, which activate the PI3k/Akt pathway, driving the proliferation, migration, and invasion of prostate cancer cells (Olivas and Price, 2021; Noda et al., 2015).

Glioblastoma is the most prevalent and aggressive malignant brain tumor in adults (Koshy et al., 2012). Although its association with obesity is not yet fully understood, some studies suggest that obesity may correlate with a poorer prognosis in patients with glioblastoma (Chambless et al., 2012). Chronic low-grade inflammation, a hallmark of obesity, characterized by the activation of inflammatory cells and the secretion of inflammatory mediators promotes glioblastoma proliferation, angiogenesis, and invasion (Sowers et al., 2014; Fossati et al., 1999; Murat et al., 2009). In certain glioblastoma cell lines, leptin - an adipokine whose levels are elevated in obesity has been found to activate several intracellular signaling pathways, including NF- κ B, p38-MAPK, and JAK/STAT3, all of which drive increased proliferation, migration and invasion (Panza et al., 2020; Yeh et al., 2009). Furthermore, elevated levels of IGF-1, commonly observed in obesity, bind to its receptor, triggering the PI3K/AKT pathway, which plays a critical role in glioblastoma progression by promoting cell proliferation and migration (Tirrò et al., 2020; Hägerstrand et al., 2010; Schlenka-Lange et al., 2008). Because there are so few scientific studies on this subject, more research is needed to clarify the underlying mechanisms and potential therapeutic implications of this association.

Surgery, chemotherapy, radiation therapy, immunotherapy, hormone therapy, and targeted therapy are frequently used in the treatment of cancer (Baudino T, 2015). However, each strategy has limitations. Surgery is only effective when the disease is detected at an early stage, radiation therapy may damage healthy cells and chemotherapy often leads to drug resistance as cancer cells become unresponsive to previously successful treatments (Kaur et al., 2023). These challenges show how urgently new therapeutic approaches are needed to overcome these limitations. Natural compounds have recently been investigated for use in combination with radiotherapy, because those compounds have antioxidant and pro-immune properties, favourable safety profile, potential as radiosensitizer and capacity to protect normal tissues (Nisar et al., 2022).

3 Betulinic acid as a treatment for obesity-related disorders

BA, also known as 3-beta-hydroxy-lup-20(29)-en-28-oic acid is sourced from the bark of the birch tree (*Betula* sp.), sycamore, eucalyptus and the plane tree (*Platanus* sp.), or it can be synthesized via chemical or enzymatic oxidation of betulin (Csuk, 2014).

BA is known for its multiple biological properties including antiviral, anti-bacterial, anti-cancer, and anti-inflammatory properties, and has been recently suggested as a way to reduce the accumulation of abdominal fat (De Melo et al., 2009; Kim et al., 2019; Pavlova et al., 2003; Schühly et al., 1999; Fulda and Debatin, 2000).

A characteristic of obesity is the presence of low-grade chronic inflammation, which is especially evident through elevated levels of systemic inflammatory markers (Karczewski et al., 2018). When inflammation becomes uncontrolled, it can contribute to several health conditions, such as autoimmune disorders, asthma, inflammatory bowel disease, and cardiovascular issues (Oliveira-Costa et al., 2022). BA inhibits the production of NO, an inflammatory mediator that contributes to certain inflammatory diseases, and phospholipase A2 activity, underscoring its role as an anti-inflammatory agent (Boparai et al., 2017; Yun et al., 2003; Bernard et al., 2001). Furthermore, in human peripheral blood mononuclear cells activated by LPS, BA suppresses inflammatory responses by modulating extracellular signal-regulated kinase (ERK), Akt, and NF- κ B signalling (Viji et al., 2011). Therefore, BA emerges as a promising candidate for diminishing the adverse effects of obesity.

3.1 Effects of betulinic acid on diabetes

As a metabolic disorder, diabetes is characterized by impairments in insulin production and disruptions in macronutrient metabolism, including carbohydrates, lipids, and proteins (Boparai et al., 2017; Riya et al., 2014). *In vivo* studies show that BA modulates key metabolic pathways involved in glucose absorption and uptake, endogenous glucose production and insulin sensitivity and resistance, making it beneficial for the treatment of T2DM (De Melo et al., 2009; Ríos and Máñez, 2018; Castro et al., 2014; Kim SJ. et al., 2014).

In vitro experiments show that BA effectively reduces α -amylase and α -glucosidase activity (Kumar et al., 2013). Thus, reducing the intestinal absorption of glucose by enzymes responsible for polysaccharides hydrolysis, there is a decreased release of free glucose into circulation (Ríos and Máñez, 2018; Kumar et al., 2013). Furthermore, treatment of β -pancreatic cells with BA induces calcium influx through a multifaceted mechanism involving ATP-dependent potassium channels and calcium-dependent chloride channels, collectively contributing to its stimulation of insulin secretion (Esievo et al., 2024; Castro et al., 2018). Additionally, using a high-fat diet-fed mice model, BA orally administered promotes glycogen synthesis by activation of the p-adenosine 5'-monophosphate-activated protein kinase (AMPK) pathway, which suppresses the expression of key gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), thereby decreasing hepatic production of glucose (Ríos and Máñez, 2018; Kim SJ. et al., 2014).

Numerous experimental studies have explored the beneficial effect of BA on diabetes. Xie et al. reported that BA was intragastrically administered to diabetic Sprague–Dawley rats, which significantly reduced blood glucose and insulin levels, suggesting its protective role in managing diabetes (Xie et al., 2017). Similarly, Salau et al. proved in an *in vitro* study that BA inhibited the activity of α -amylase and α -glucosidase (Salau et al., 2024). Furthermore, Kim, et al. observed that gene expression of gluconeogenic proteins (PEPCK and G6Pase) was inhibited by BA treatment in HepG2 cells, thereby reducing hepatic glucose production (Kim SJ. et al., 2014). Moreover, findings from these studies also revealed that BA increased the phosphorylation of

AMPK in HepG2 cells, indicating AMPK activation as an important pathway in the suppressive effect of BA (Kim SJ. et al., 2014).

Despite its promising antidiabetic effects, a study by Ahangarpour, et al. reported adverse outcomes. They observed that oral administration of BA via gavage for 2 weeks in STZ–nicotinamide diabetic mice induced adverse reproductive effects, such as lower testosterone, reduced sperm counts, and histopathological deterioration of the seminiferous tubules (Ahangarpour et al., 2016). Most research on BA's anti-diabetic effects has been conducted in rodent models, which may not fully translate to the complex human pathophysiology of T2DM. In addition, evidence also showed that very high doses of BA (2,000 mg/kg) caused temporary anaemia in rats. Although the effect was reversed later, it suggests that high doses of BA could worsen anaemia in diabetic patients, highlighting the importance of cautious dosing and further safety testing (Esievo et al., 2023).

3.2 Effects of betulinic acid on cardiovascular diseases

BA has gained attention as a potential therapeutic agent for managing CVD due to its ability to diminish the progression of atherosclerotic lesions and myocardial dysfunction (Silva et al., 2016). Atherosclerosis begins with vascular inflammation, driven by damage from atherosclerotic plaques, which may contribute to the development of cardiovascular complications (Yoon et al., 2017; Choi et al., 2003). *In vitro* studies showed that BA counteracts this process by suppressing NF- κ B activation and ROS production, which reduces inflammatory cells recruitment (Yoon et al., 2010). BA also improves endothelial function by decreasing expression of adhesion molecules like ICAM-1, VCAM-1, E-selectin, and endothelin-1 (ET-1), as demonstrated in apolipoprotein E knockout (ApoE KO) mice administered oral BA, all of which are implicated in the progression of coronary atherosclerosis (Yoon et al., 2017). Additionally, treatment of BA in endothelial cells promotes vasorelaxation by enhancing NO production via endothelial nitric oxide synthase (eNOS) activation, driven by the AMPK pathway (Yoon et al., 2017; Jin et al., 2016). Some examples demonstrating BA's effects on CVD include a study by Afghan, et al. showing that a high-fat diet in BALB/c mice led to elevated levels of total cholesterol, triglycerides, and LDL, indicative of atherosclerotic changes (Afghan et al., 2022). This investigation demonstrated that BA was administered orally and effective in reducing total cholesterol, LDL, VLDL, and triglyceride levels while increasing HDL levels in BALB/c mice, underscoring the protective role of BA against CVD (Afghan et al., 2022). Other case is the study report by, Yoon, et al., in which orally administered BA was found to reduce atherosclerotic lesions and to prevent the decrease of eNOS expression in ApoE gene-deficient C57BL/6J mice, further supporting its anti-atherosclerotic properties (Yoon et al., 2017). Despite the potential positive properties of BA, several limitations restrict its use in cardiovascular disease treatment. BA has very low oral bioavailability, with less than 1% of the administered dose absorbed into circulation, which limits its therapeutic effectiveness (Godugu et al., 2014). Furthermore, clinical trials directly evaluating BA in cardiovascular patients are lacking, so its safety profile in this

context remains uncertain (Silva et al., 2016). While BA has not been directly linked to cardiovascular toxicity, the absence of human studies and the possibility of adverse effects at higher doses raise important safety concerns for its use in cardiovascular therapy.

3.3 Effects of betulinic acid on nonalcoholic fatty liver disease

BA also has the potential to be a therapeutic agent for NAFLD. The compound works as an agonist of the farnesoid X receptor (FXR), which is a critical regulator of lipid and glucose metabolism, inflammatory responses, and the liver's energy homeostasis (Yao and Liu, 2022; Gu et al., 2019). Through FXR activation, BA mitigates hepatic ER stress *in vitro* by suppressing the PERK/EIF2 α /ATF4 signalling pathway, which is known to contribute to hepatic steatosis (Gu et al., 2019; Wang et al., 2023). FXR activation also decreases lipotoxicity, promotes cholesterol metabolism, improves insulin resistance, and exerts anti-inflammatory effects (Gu et al., 2019). Furthermore, BA regulates fat metabolism by reducing lipid accumulation and fat synthesis, while stimulating fatty acid oxidation, in human hepatocyte-derived cells (Quan et al., 2013). Additionally, Mu, et al. reported that BA, administered either by oral gavage or incorporated into a high-fat diet, suppresses the activity of fatty acid synthase (FAS), by downregulating the expression of Yin Yang 1 (YY1), a key upstream regulator of FAS (Mu et al., 2020). BA has also been shown to attenuate liver fibrosis and represents a promising candidate for the development of anti-fibrotic therapies aimed at preventing NASH (Mu et al., 2020).

Some studies have explored the association between BA and NAFLD. Gu, et al. demonstrated that BA acts as a selective activator of FXR in HEK293T cells and that C57BL/6J diet-induced obesity mice, receiving BA mixed into their high-fat diet, exhibited lower levels of hepatic steatosis, as evidenced by a decrease in hepatic lipid content, along with decreased expression of hepatic ER stress-related genes, indicating BA's ability to attenuate ER stress during hepatic steatosis (Gu et al., 2019). Moreover, they analysed liver damage markers, such as ALT and AST, finding that BA treatment significantly lowered ALT levels but not AST levels, suggesting an improvement in hepatic steatosis in C57BL/6J diet-induced obesity mice (Gu et al., 2019). Furthermore, Zhao et al. investigated the anti-fibrotic effects of BA and proved that C57BL/6J mice with hepatic fibrosis, treated daily with BA by oral gavage, exhibited lower collagen amounts in liver tissues compared to untreated controls, confirming that BA attenuates liver fibrosis (Zhao et al., 2022).

Although BA shows promising effects in NAFLD models, current evidence is primarily preclinical, and there are no direct reports of toxicity in these studies. However, potential risks at higher doses cannot be ruled out, and some data from other models suggest dose-dependent cytotoxicity in non-target cells (Yao and Liu, 2022). Moreover, gaps remain regarding its pharmacokinetics, long-term safety, and possible off-target effects in humans. Therefore, clinical studies are needed to confirm both the efficacy and safety of BA before it can be recommended as a therapy for NAFLD.

3.4 Effects of betulinic acid on cancer

The anti-cancer effect of BA rises essentially from its capacity to induce apoptosis. Apoptosis, or programmed cell death, proceeds via two main routes: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway (Kiraz et al., 2016). The mitochondrial pathway is regulated by anti-apoptotic proteins, such as members of the Bcl-2 family, and pro-apoptotic proteins like Bax (Mullauer et al., 2010). *In vitro* studies demonstrate that BA promotes the mitochondrial pathway, promoting the permeabilization of the outer mitochondrial membrane (MOMP), which results in the release of cytochrome c and Smac into the cytosol, ultimately triggering the activation of cytosolic caspase (Fulda, 2008; Kumar et al., 2018; Wang et al., 2017). Furthermore, BA enhances the expression of proapoptotic proteins while suppressing antiapoptotic proteins in colorectal cancer cells, which promotes apoptosis (Zeng et al., 2019).

In cancer cells apoptosis induced by BA occurs through the increase in ROS production, a process that is also elevated in obesity, and contributes to the initiation of MOMP (Kumar et al., 2018; Csuk et al., 2010). Additionally, the activation of the NF- κ B pathway is a characteristic of many cancer types, driving epigenetic changes, EMT, angiogenesis, metastasis, drug resistance, and immunosuppression (Jiang et al., 2021). The phosphorylation of I κ B proteins is driven by increased activity of the inhibitor of nuclear factor- κ B kinase (IKK) followed by the degradation of the IKK complex itself (Jiang et al., 2021; Shankar et al., 2017). This releases NF- κ B dimers, which translocate to the nucleus, where p65 subunit activates the NF- κ B, binding to specific DNA sequences and upregulating target genes involved in proliferation and anti-apoptotic signalling (Jiang et al., 2021; Shankar et al., 2017). *In vitro* studies have shown that BA inhibits these effects by reducing nuclear levels of NF- κ B/p65, inhibiting IKK activity, and stimulating I κ B α phosphorylation at serine 32/36, leading to its degradation (Lou et al., 2021; Rabi et al., 2008). Therefore, the inhibition of the NF- κ B signaling pathway by BA, induces apoptosis and the suppression of cell proliferation (Su et al., 2017). These mechanisms are represented in Figure 3.

BA's anti-cancer effect may also rely on the inhibition of angiogenesis and metastasis. BA has been shown to reduce the expression of Sp transcription factors (Sp1, Sp3, Sp4) in breast cancer cells, suppressing VEGF and its receptor (VEGFR), which prevents new blood vessel formation (Luo et al., 2016; Yang et al., 2014). In addition, BA suppresses HIF-1 α levels by inhibiting prolydase (an enzyme central to angiogenic signalling) and reduces integrins expression in endometrial cancer cells, inhibits aminopeptidase N activity *in vitro* and suppresses signal transducer and activator of transcription 3 (STAT3) expression in prostate cancer cells (Karna et al., 2010; Shin et al., 2011; Melzig and Bormann, 1998). In what concerns anti-metastatic activity, BA reduces the expression of matrix metalloproteinase MMP-2 and MMP-9 while increases tissue inhibitor of metalloproteinase 2 (TIMP2) in breast cancer cells and E-cadherin levels in gastric cancer cells (Amiri et al., 2020; Zeng et al., 2017; Chen et al., 2020).

Evidence shows that BA is effective in the treatment of many cancers associated with obesity, such as breast cancer, prostate cancer, colorectal cancer, endometrial cancer, glioblastoma and

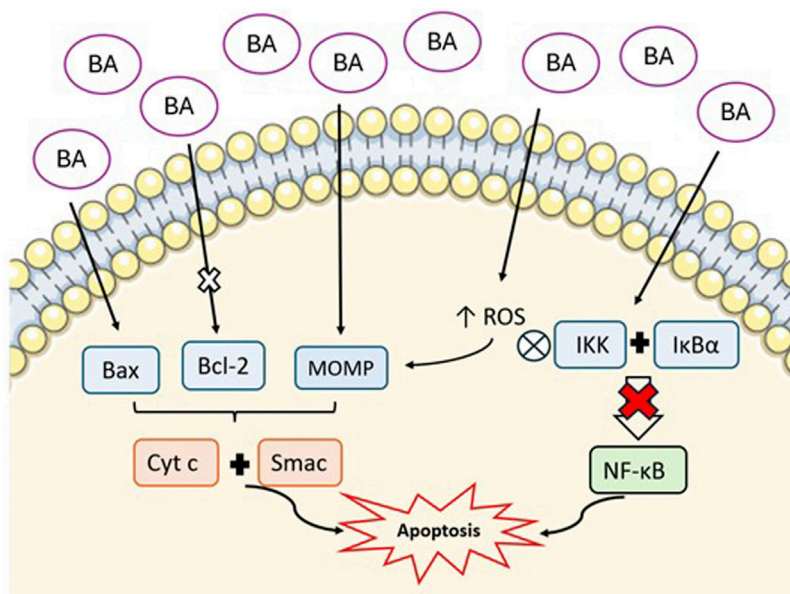


FIGURE 3
Representation of the mechanism of BA-induced apoptosis via mitochondrial pathway. BA increases pro-apoptotic proteins (Bax), inhibits anti-apoptotic proteins (Bcl-2), and promotes mitochondrial outer membrane permeabilization (MOMP). This results in the release of cytochrome c (Cyt c) and Smac into the cytosol. Additionally, BA increases reactive oxygen species (ROS) and inhibits NF- κ B pathway by suppressing IKK complex and I κ B α . All these mechanisms culminate in the promotion of apoptosis. (Image created with an icon from smart.servier.com).

many others (Ali-Seyed et al., 2016; Tiwari et al., 2014; Chintharlapalli et al., 2007; Aisha et al., 2012; Karna and Palka, 2009; Bache et al., 2011).

In breast cancer, BA leads to the arrest of the cell cycle on the G2/M phase and promotes cell apoptosis through the mitochondrial pathway in MCF-7 cells by downregulating the level of expression of Bcl-2 while upregulating the level of expression of Bax (Sun et al., 2013). In addition, BA inhibited angiogenesis in MDA-MB-453 and BT474 cells by repressing the levels of Sp1, Sp3, and Sp4 via disruption of microRNA-27a (Liu et al., 2012). Weber, et al. observed that BA inhibited NF- κ B activation and downregulated TNF- α , STAT3, and i-NOS expression in MDA-MB-231 and MDA-MB-468 cells, demonstrating that BA has anti-inflammatory effects (Weber et al., 2014). Finally, BA exerts also anti-metastatic activity by reducing MMP-2 and MMP-9 secretion while at the same time upregulating TIMP2 in 4T1 and MDA-MB-231 cells (Zeng et al., 2017).

BA has shown potential as a therapeutic agent for prostate cancer, as it affects selectively cancer cells and leaves the normal cells unharmed (Reiner et al., 2013). In prostate cancer cell lines LNCaP and PC3, BA promotes the degradation of pro-survival proteins, including the androgen receptor (AR), which is significant because AR signalling strongly influences the progression of prostate cancer, while normal cells remain unaffected (Reiner et al., 2013). Shankar et al. demonstrated that BA suppressed cell viability and stimulated apoptosis in LNCaP and DU145 cells (Shankar et al., 2017). Key mechanisms driving cancer progression include dysregulated NF- κ B activity and p53 inactivation, which is involved in programmed cell death responses to DNA damage in many cancer cells (Shankar et al., 2017; Schneider et al., 2010; Murphy et al., 2011). Additionally, the same authors showed that BA inhibited the NF- κ B pathway in

LNCaP and DU145 cells by reducing phosphorylation of I κ B α at Ser32/36, thereby enhancing its stability and preventing the nuclear translocation and DNA binding activity of NF- κ B/p65, ultimately triggering apoptosis (Shankar et al., 2017).

The impact of BA on colorectal cancer has also been extensively studied. Chintharlapalli, et al. found out that BA inhibits the proliferation of cancer cells by promoting the suppression of Sp1, Sp3, and Sp4 in SW480 and RKO colorectal cancer cell lines (Chintharlapalli et al., 2011). Zeng et al. further observed that BA decreases cell growth in HCT116 cells by downregulating BCL-2, upregulating Bax, and disrupting mitochondrial membrane potential, while simultaneously increasing ROS production (Zeng et al., 2019). These findings suggest that apoptosis induced by BA in colorectal cancer cells may be mediated by mitochondria (Zeng et al., 2019). Additionally, research carried out by Su, et al. showed that BA also reduces proliferation and induces apoptosis in HT-29 cells (Su et al., 2017). Furthermore, Zeng, et al. also revealed that BA-treated HCT116 cells showed repressed migration and invasion ability. This was confirmed by significantly altered levels of MMP-2 and MMP-9 expression, along with reduced TIMP-2 levels (Zeng et al., 2019).

Some studies have looked at the role of BA on endometrial cancer. Karna and Palka showed that BA was able to induce a 50% reduction in viability of Ishikawa endometrial cancer cell line (Karna and Palka, 2009). Furthermore, Karna, et al. demonstrated that BA reduced both collagen production and prolydase activity, an enzyme involved in collagen degradation and angiogenic signalling pathways, in Ishikawa cells (Karna et al., 2010). Additionally, Ishikawa cells treated with BA expressed decreased levels of HIF-1 α , which is a key factor in angiogenesis, and VEGF (Karna et al., 2010). Furthermore, BA downregulated the expression of α 1 and

$\alpha 2$ integrin subunits, thus indicating that BA can interfere with the angiogenic process through HIF-1 α , VEGF, and integrin signaling pathways in these cells (Karna et al., 2010).

Beyond the antitumor effects of BA on epithelial cancers, it has been shown to induce apoptosis in non-epithelial cancers (Fulda et al., 1999). Some examples are glioblastoma and neuroectodermal tumour cells, where activation of the caspase cascade has an important role (Fulda et al., 1999; Fulda et al., 1997). In glioblastoma, the antitumour role of BA has been extensively studied, particularly its impact on cell proliferation, migration, and apoptosis (Fernandes et al., 2024; Lo et al., 2020). Yaozu et al. observed that BA reduced the proliferative potential of glioblastoma cell lines A172 and U87MG while promoting apoptosis (Yaozu et al., 2020). This study further revealed that BA inhibited NF- κ B activation, by decreasing intracellular levels of NF- κ B p65 in A172 and U87MG cell lines, reduced BCL-2 levels suppressed the levels of the X-linked inhibitor of apoptosis protein (XIAP), and increased the expression of pro-apoptotic markers such as BAX, caspase-3, and caspase-9, indicating that BA promotes apoptosis by suppressing the NF- κ B signalling pathway (Yaozu et al., 2020). Moreover, Wei-Lun, et al. showed that BA enhances the therapeutic efficacy of temozolomide (TMZ) in glioblastoma, particularly in TMZ-resistant cells (Lo et al., 2020). TMZ, normally used for glioblastoma treatment, combined with BA was shown to inhibit glioblastoma progression, with BA suppressing the expression of Sp1 indicating a potential combined therapeutic strategy (Lo et al., 2020). In contrast, the impact of BA on angiogenesis and metastasis in glioblastoma has not been fully explored, highlighting the need for further investigations into these mechanisms, particularly its effects on VEGF, MMP-2, and MMP-9.

Obesity is also known to contribute to the progression of melanoma, and the potential therapeutic role of betulinic acid (BA) in this context has also been investigated (Dobbins et al., 2013). Coricovac et al. showed that BA reduced the viability of A375 human melanoma cells by inducing mitochondrial dysfunction and promoting apoptosis (Coricovac et al., 2021). Similarly, Liu et al. reported that BA increases ROS production and disrupts mitochondrial membrane potential in B16 murine melanoma cells, resulting in apoptosis (Liu et al., 2004). Expanding on these findings, Tan et al. showed that BA induces apoptosis in UISO-Mel-1 melanoma cells through the activation of pro-apoptotic MAPK signaling pathways, particularly p38 and SAP/JNK (Tan et al., 2003). In another study, BA significantly reduced the proliferation of ME20, ME21, A375, 518A2 melanoma cells and exhibited an additive effect when combined with radiotherapy (Selzer et al., 2000). Furthermore, Gheorgheosu et al. demonstrated that BA suppresses EMT in A375 melanoma cells by downregulating mesenchymal markers and upregulating epithelial markers, highlighting its potential to counteract melanoma metastasis (Gheorgheosu et al., 2013).

The therapeutic impact of BA on lung cancer, which can be exacerbated by central obesity, has also been investigated (Gao et al., 2019). Hsu et al. demonstrated that BA inhibits the transcription factor Sp1 in A549 and H1299 lung cancer cells, leading to reduced expression of cyclin A2, resulting in cell cycle arrest at the G2/M phase, and ultimately inhibition of cell proliferation (Hsu et al., 2012). Additionally, Zuco et al. reported that BA effectively reduces

the proliferation of H460 and POGB lung cancer cells while exhibiting significantly lower toxicity toward normal cells, highlighting its potential as a safer anticancer agent (Zuco et al., 2002). Kessler et al. further observed that BA induced significant cell death in the A549, H187, N417, and MBA9812 lung cancer cell lines, with viability dropping below 10% after 48 h of treatment, suggesting strong cytotoxic effects in both non-small and small cell lung cancer types (Kessler et al., 2007). Furthermore, another study reported that BA induces apoptosis in paclitaxel-resistant H460 lung cancer cells by reducing cell viability, disrupting mitochondrial membrane potential, and increasing the Bax/Bcl-2 ratio, which highlights its potential to overcome chemoresistance (Zhan et al., 2018).

BA has demonstrated significant effects on pancreatic cancer growth, a malignancy closely linked to obesity (Genkinger et al., 2011). Guo et al. studied the effect of BA on PANC-1 and SW1990 pancreatic cancer cell lines and observed that BA reduced cell viability in a dose-dependent manner and effectively induced apoptosis through activation of the AMPK/mTOR signaling pathway (Guo et al., 2020). Additionally, the study by Sun et al. showed that BA suppresses this migration and invasion of Mia PaCa-2 and PANC-1 cells by inhibiting EMT, as evidenced by increased E-cadherin expression and reduction of vimentin levels (Sun et al., 2018). In another study, BA was also shown to significantly inhibit the viability of Mia PaCa-2 and SUIT-2 pancreatic cancer cells, at low concentrations, while sparing normal pancreatic cells, which suggests its therapeutic impact in pancreatic cancer (Chiu et al., 2021). This study further showed that BA upregulates genes involved in metabolism and tumor suppression, while downregulating genes associated with inflammation and cell migration, suggesting a role in reducing the migratory capacity of PDAC cells (Chiu et al., 2021).

Obesity increases the risk of ovarian cancer, a disease in which the effects of BA have also been investigated (Bander et al., 2016). In the study by Lee et al., BA reduced the viability of A2780 human ovarian carcinoma cells in a dose-dependent manner, demonstrating significant cytotoxic effects (Lee et al., 2019). Additionally, BA induced apoptosis, as evidenced by increased activation of caspases-3, -8, and -9, upregulation of Bax, and downregulation of Bcl-2, indicating involvement of both mitochondrial and death receptor pathways (Lee et al., 2019). In another study by Liao et al., BA also suppressed the proliferation of SKOV3 and SW626 ovarian cancer cells, while exhibiting minimal cytotoxicity toward normal cells (Liao et al., 2020). Furthermore, BA significantly inhibited cell migration and invasion by blocking EMT, as shown by decreased N-cadherin and increased E-cadherin expression (Liao et al., 2020). *In vivo*, intraperitoneal administration of BA reduced tumor volume and weight in a SKOV3 xenograft mouse model and downregulated Ki-67 and MMP-2 expression, supporting its anti-metastatic and anti-proliferative activity (Liao et al., 2020).

All these findings prove BA's efficacy as a therapeutic agent for several obesity-related disorders. The summary of BA effects on those diseases is presented in Table 1.

Regarding the effect of BA on cancer, some contradictory information has been reported. Although BA often inhibits NF- κ B to suppress cancer cell proliferation, it can also activate NF- κ B in a cell-type-specific manner (Fulda, 2008). In some cancer cells, this

TABLE 1 Summary of BA's effect on obesity-related disorders.

Obesity-related disorder	<i>In vitro/in vivo</i> models	Effects of BA	References
Diabetes	HepG2 cells Sprague-Dawley rats	↓ Glucose levels ↓ Insulin levels ⊗ α -amylase ⊗ α -glucosidase ⊗ gluconeogenic proteins ↑ AMPK activation	Kim et al. (2014a) Xie et al. (2017) Salau et al. (2024)
Cardiovascular diseases	BALB/c mice C57BL6J mice	↓ total cholesterol levels ↓ LDL levels ↓ VLDL levels ↓ triglycerides levels ↑ HDL levels ↓ atherosclerotic lesions	Yoon et al. (2017) Afghan et al. (2022)
NAFLD	HEK293T cells C57BL/6J mice	↓ Hepatic steatosis ↑ Hepatic lipid content ↓ ALT levels ↓ Hepatic ER stress ↓ Liver fibrosis	Gu et al. (2019) Zhao et al. (2022)
Breast Cancer	MCF-7 cells MDA-MB-468 cells MDA-MB-231 cells BT474 cells 4T1 cells	↑ Apoptosis ⊗ Angiogenesis ⊗ Inflammation ⊗ Metastasis	Sun et al. (2013) Liu et al. (2012) Weber et al. (2014) Zeng et al. (2017)
Prostate Cancer	LNCaP cells PC3 cells DU145 cells	Degradation of AR ↓ Cell viability ⊗ NF- κ B pathway ↑ Apoptosis	Reiner et al. (2013) Shankar et al. (2017) Schneider et al. (2010) Murphy et al. (2011)
Colorectal Cancer	SW480 cells RKO cells HCT116 cells HT-29 cells	⊗ Cell proliferation ↓ Cell growth ↑ Apoptosis ⊗ Migration and invasion	Zeng et al. (2019) Su et al. (2017) Chintharlapalli et al. (2011)
Endometrial Cancer	Ishikawa cells	↓ Cell viability ↓ Collagen production ↓ Prolidase activity ⊗ Angiogenesis	Karna and Palka (2009) Karna et al. (2010)
Glioblastoma	A172 cells U87MG cells Balb/c mice Sprague-Dawley rats	↑ Apoptosis ⊗ NF- κ B activation ↓ Bcl-2 ↑ Bax, caspase-3, caspase 9 ⊗ Sp1 Penetrates BBB	Lo et al. (2020) Yaozu et al. (2020) Killinger et al. (2014) Li et al. (2022)
Melanoma	A375 cells B16 cells UIISO-Mel-1 cells ME20 cells ME21 cells 518A2 cells	↓ Cell viability ↑ Apoptosis ↑ ROS production ↓ Cell proliferation ⊗ EMT	Dobbins et al. (2013) Coricovac et al. (2021) Liu et al. (2004) Tan et al. (2003) Selzer et al. (2000) Gheorgheosu et al. (2013)
Lung Cancer	A549 cells H1299 cells H460 cells POGB cells H187 cells N417 cells MBA9812 cells	⊗ Sp1 ↓ Cell proliferation ↑ Cell death ↓ Cell viability ↑ Apoptosis ↑ Bax/Bcl-2	Hsu et al. (2012) Zuco et al. (2002) Kessler et al. (2007) Zhan et al. (2018)
Pancreatic Cancer	PANC-1 cells SW1990 cells Mia PaCa-2 cells SUIT-2 cells	↓ Cell viability ↑ Apoptosis ⊗ Migration and invasion ⊗ EMT ↑ E-cadherin ↓ Vimentin ↑ Genes involved in metabolism and tumor suppression ↓ Genes associated with inflammation and cell migration	Guo et al. (2020) Sun et al. (2018) Chiu et al. (2021)

(Continued on following page)

TABLE 1 (Continued) Summary of BA's effect on obesity-related disorders.

Obesity-related disorder	<i>In vitro/in vivo</i> models	Effects of BA	References
Ovarian Cancer	A2780 cells SKOV3 cells SW626 cells SKOV3 xenograft mouse	↓ Cell viability ↑ Apoptosis ↑ caspase 3, 8, 9 ↑ Bax ↓ Bcl-2 ↓ Cell proliferation ⊗ Migration and invasion ⊗ EMT ↓ Tumor volume and weight ↓ Ki-67 ↓ MMP-2	Lee et al. (2019) Liao et al. (2020)

⊗ - inhibition; ↑ - increase; ↓ - reduction.

activation contributes to BA-induced apoptosis, as blocking NF- κ B reduces apoptotic effects (Fulda, 2008). These findings indicate that BA's impact on NF- κ B is context-dependent, highlighting the complexity of its anticancer mechanisms. Although BA is generally reported to spare normal cells, several studies indicate that at high concentrations or prolonged exposure, BA can induce cytotoxicity in normal cells. For example, murine and human fibroblasts can exhibit reduced viability and mitochondrial dysfunction at BA doses near those effective in cancer cells, indicating context-dependent selectivity (Małaczewska et al., 2021).

3.5 Challenges and limitations in the therapeutic use of betulinic acid

BA displays poor pharmacokinetic properties, largely due to its extremely low aqueous solubility, which limits gastrointestinal absorption to less than 1%, resulting in minimal systemic bioavailability (Jiang et al., 2021; Jäger et al., 2009). Experimental studies in rodents confirm high plasma protein binding and uneven tissue distribution, with substantial accumulation in organs such as lymph nodes, ovaries, and liver, while circulating levels remain consistently low. In mice, BA intraperitoneally administered at doses of 250 or 500 mg/kg had elimination half-lives of 11.5 and 11.8 h, and total clearances of 13.6 and 13.5 L/kg/h, where serum concentrations peaking at 0.15 and 0.23 h, respectively (Ríos and Máñez, 2018; Jäger et al., 2009). These limitations complicate both *in vitro* and *in vivo* experimental designs and restrict the compound's therapeutic potential. To address these issues, researchers have explored different strategies, including polymer complexes, mucoadhesive microparticles, nanocarriers, and structural modifications. Although these approaches have improved solubility and significantly enhanced oral bioavailability in experimental models, they remain at a preclinical stage (Ríos and Máñez, 2018).

Metabolic studies further show that BA undergoes transformation through human CYP enzymes, with C-28, C-6, and C-23 being the main sites of oxidative metabolism. While chemical modifications at specific positions have been shown to improve solubility without compromising activity, novel delivery strategies such as liposomes, nanoemulsions, transdermal systems, and sustained-release formulations are also under investigation (Godugu et al., 2014; Wang et al., 2024).

Even though BA demonstrates considerable therapeutic potential for several diseases, this compound has other several limitations. A central problem is BA's extraction and production, because the direct extraction method produces lower quantities and chemical synthesis demands strict reaction conditions (Jiang et al., 2021; Jäger et al., 2009). For example, extraction with 70% ethanol yielded 23.76 mg of BA per 10 g of birch bark, whereas ultrasonic extraction produced only 0.0021% (Mukherjee et al., 2010; Kim H-I. et al., 2014). Particularly, regarding brain cancers treatment, the cross of the blood-brain barrier (BBB) is still an obstacle for therapeutic agents. This happens because of the presence of tight junctions and active transport mechanisms and the selectivity of this barrier (Fernandes et al., 2024). Interestingly, BA can cross the BBB, suppressing one of the primary obstacles to treating glioblastoma and other neurological disorders (Killinger et al., 2014).

One promising strategy to address BA's limited access to brain tissue is the development of BA micro or nanoparticles, as is the case of hybrid polymer nanoformulations with targeted ligands, which enhances its stability, sustained release and safety (Li et al., 2022). Their biochemical versatility allows them to integrate multiple properties from different materials into a single micro or nanoformulation, enhancing their therapeutic potential (Roque et al., 2023). In preclinical studies, nanoparticles successfully penetrated the brains of mice, reducing ischemia-induced infarction and demonstrating significant antitumoral effects in glioblastoma models (Li et al., 2022; Deng et al., 2019). Despite promising advances, the development of effective administration routes and stable formulations remains one of the greatest challenges to translating BA into clinical application.

Another important approach is the use of ionic liquid formulations, which increase the aqueous solubility of BA and improve its therapeutic action (Suresh et al., 2012). In fact, some studies have demonstrated that synthesized organic salts and BA ionic derivatives exhibit stronger antiproliferative activity and higher cytotoxicity against various cancer cell lines (Suresh et al., 2012; Silva et al., 2019). Furthermore, in another study, novel indole-functionalized BA derivatives have exhibited high cytotoxicity against several cancer cell lines, indicating their potential as promising candidates for cancer treatment (Fernandes et al., 2024; Bębenek et al., 2022).

Regarding BA's toxicity, evidence suggests that spray-dried (SD) formulations of BA, caused no changes in body weight in animal models, and histopathology of the intestine, liver, lung, heart, spleen, and kidney showed no tissue damage (Godugu et al., 2014). Furthermore, BA has some adverse effects, including very low

oral bioavailability which, together with the scarcity of clinical trials in humans, leaves its efficacy and long-term safety in cardiovascular, metabolic, hepatic, or oncological contexts uncertain. Preclinical studies report dose-dependent cytotoxicity in non-target cells, temporary anaemia at high doses, and reproductive toxicity in diabetic mice. Clinical trials using BA reached phase I/II and almost none of them have progressed to phase III (Ruiz-Molina et al., 2022; Gielecińska et al., 2023). Those studies have often stalled at the animal model testing stage, caused mainly by the lack of evidence concerning the safety of these drugs, potential mid-to long-term toxicity, immunogenicity, and pharmacokinetic profile. Furthermore, BA's effects on pathways such as NF- κ B are context-dependent, showing both inhibitory and activating actions depending on the cell type, which may influence therapeutic outcomes and adverse effects. These results highlight the need for additional preclinical and clinical evaluation to assess the safety and efficacy of BA.

4 Main conclusion and future directions

Obesity is a widespread condition that impacts many individuals globally and is associated with an increased risk of several health issues, including diabetes, CVD, NAFLD, and cancer. This review compiles the information on BA use, which shows great therapeutic potential for the management of those obesity-related disorders. Overall, the compound exhibits antioxidant, anti-inflammatory, and antitumoral effects, as well as impacting glucose and lipid metabolism, apoptosis, angiogenesis and metastasis.

Despite BA showing great potential in laboratory studies, clinical results are still in the early stages, where the efficacy, safety and the best protocols of the compound are being explored. Unlocking the full therapeutic potential of BA could open the way for transformative advancements in treating obesity-related diseases, offering hope for millions worldwide.

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Author contributions

LA: Conceptualization, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review and editing. RF: Formal Analysis, Investigation, Methodology, Project

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References

- Abdelmalek, M. F., Suzuki, A., Guy, C., Unalp-Arida, A., Colvin, R., Johnson, R. J., et al. (2010). Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology* 51 (6), 1961–71. doi:10.1002/hep.23535
- Afghan, S., Jahan, S., Zainab, A., Khan, M., Aslam, H., and Ahmad, S. (2022). Effect of betulinic acid on lipid homeostasis and atherosclerotic index. *Int. J. Pathol.* 20 (1), 14–18.
- Ahangarpour, A., Oroojan, A. A., Khorsandi, L., Arzani, G., and Afshari, G. (2016). Effects of betulinic acid on the Male reproductive system of a streptozotocin-nicotinamide-induced diabetic mouse model. *World J. men's health* 34 (3), 209–16. doi:10.5534/wjmh.2016.34.3.209
- Aisha, A. F., Abu-Salah, K. M., Ismail, Z., and Majid, AMSA (2012). alpha-Mangostin enhances betulinic acid cytotoxicity and inhibits cisplatin cytotoxicity on HCT 116 colorectal carcinoma cells. *Molecules* 17 (3), 2939–54. doi:10.3390/molecules17032939
- Al-Goblan, A. S., Al-Alfi, M. A., and Khan, M. Z. (2014). Mechanism linking diabetes mellitus and obesity. *Diabetes, metabolic syndrome Obes. targets Ther.* 7, 587–91. doi:10.2147/DMSO.S67400
- Ali-Seyed, M., Jantan, I., Vijayaraghavan, K., and Bukhari, S. N. (2016). Betulinic acid: recent advances in chemical modifications, effective delivery, and molecular

- mechanisms of a promising anticancer therapy. *Chem. Biol. Drug Des.* 87 (4), 517–36. doi:10.1111/cbdd.12682
- Alloubani, A., Nimer, R., and Samara, R. (2021). Relationship between hyperlipidemia, cardiovascular disease and stroke: a systematic review. *Curr. Cardiol. Rev.* 17 (6), e051121189015. doi:10.2174/1573403X16999201210200342
- Amini-Salehi, E., Letafatkar, N., Norouzi, N., Joukar, F., Habibi, A., Javid, M., et al. (2024). Global prevalence of nonalcoholic fatty liver disease: an updated review meta-analysis comprising a population of 78 million from 38 countries. *Arch. Med. Res.* 55 (6), 103043. doi:10.1016/j.arcmed.2024.103043
- Amiri, S., Dastghaib, S., Ahmadi, M., Mehrbod, P., Khadem, F., Behrouj, H., et al. (2020). Betulin and its derivatives as novel compounds with different pharmacological effects. *Biotechnol. Adv.* 38, 107409. doi:10.1016/j.biotechadv.2019.06.008
- Apovian, C. M. (2016). Obesity: definition, comorbidities, causes, and burden. *Am. J. Manag. Care* 22 (7 Suppl. 1), s176–85. Available online at: <https://www.ajmc.com/view/obesity-definition-comorbidities-causes-burden>.
- Avgerinos, K. I., Spyrou, N., Mantzoros, C. S., and Dalamaga, M. (2019). Obesity and cancer risk: emerging biological mechanisms and perspectives. *Metabolism* 92, 121–35. doi:10.1016/j.metabol.2018.11.001
- Bache, M., Zschornak, M. P., Passin, S., Kessler, J., Wichmann, H., Kappler, M., et al. (2011). Increased betulinic acid induced cytotoxicity and radiosensitivity in glioma cells under hypoxic conditions. *Radiat. Oncol.* 6, 111. doi:10.1186/1748-717X-6-111
- Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci.* 101 (44), 15718–23. doi:10.1073/pnas.0407076101
- Bandera, E. V., Qin, B., Moorman, P. G., Alberg, A. J., Barnholtz-Sloan, J. S., Bondy, M., et al. (2016). Obesity, weight gain, and ovarian cancer risk in African American women. *Int. J. Cancer* 139 (3), 593–600. doi:10.1002/ijc.30115
- Bandini, M., Gandaglia, G., and Briganti, A. (2017). Obesity and prostate cancer. *Curr. Opin. Urol.* 27 (5), 415–21. doi:10.1097/MOU.0000000000000424
- Baudino T, A. (2015). Targeted cancer therapy: the next generation of cancer treatment. *Curr. drug Discov. Technol.* 12 (1), 3–20. doi:10.2174/1570163812666150602144310
- Bębenek, E., Chrobak, E., Rzepka, Z., and Wrześniak, D. (2022). New betulin derivatives with Nitrogen heterocyclic moiety—synthesis and anticancer activity *in vitro*. *Biomolecules* 12 (10), 1540. doi:10.3390/biom12101540
- Bernard, P., Scior, T., Didier, B., Hibert, M., and Berthon, J.-Y. (2001). Ethnopharmacology and bioinformatic combination for leads discovery: application to phospholipase A2 inhibitors. *Phytochemistry* 58 (6), 865–74. doi:10.1016/S0031-9422(01)00312-0
- Berrington de Gonzalez, A., Hartge, P., Cerhan, J. R., Flint, A. J., Hannan, L., MacInnis, R. J., et al. (2010). Body-mass index and mortality among 1.46 million white adults. *N. Engl. J. Med.* 363 (23), 2211–9. doi:10.1056/NEJMoa1000367
- Booth, A., Magnuson, A., Fouts, J., and Foster, M. (2015). Adipose tissue, obesity and adipokines: role in cancer promotion. *Hormone Mol. Biol. Clin. investigation* 21 (1), 57–74. doi:10.1515/hmbci-2014-0037
- Boparai, A., Niazi, J., Bajwa, N., and Singh, P. (2017). Betulin a pentacyclic tri-terpenoid: an hour to rethink the compound. *Open Access J. Trans. Med. Res.* 1 (2), 53–9. doi:10.15406/oajtmr.2017.01.00012
- Brismar, K., Fernqvist-Forbes, E., Wahren, J., and Hall, K. (1994). Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. *J. Clin. Endocrinol. Metab.* 79 (3), 872–8. doi:10.1210/jcem.79.3.7521354
- Brown, K. A. (2021). Metabolic pathways in obesity-related breast cancer. *Nat. Rev. Endocrinol.* 17 (6), 350–63. doi:10.1038/s41574-021-00487-0
- Brun, P., Castagliuolo, I., Leo, V. D., Buda, A., Pinzani, M., Palu, G., et al. (2007). Increased intestinal permeability in Obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am. J. Physiology-Gastrointestinal Liver Physiology* 292 (2), G518–G525. doi:10.1152/ajpgi.00024.2006
- Brunner, K. T., Henneberg, C. J., Wilechansky, R. M., and Long, M. T. (2019). Nonalcoholic fatty liver disease and obesity treatment. *Curr. Obes. Rep.* 8 (3), 220–8. doi:10.1007/s13679-019-00345-1
- Cani, P. D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., et al. (2009). Changes in gut microbiota control inflammation in Obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 58 (8), 1091–103. doi:10.1136/gut.2008.165886
- Castro, A. J. G., Frederico, M. J. S., Cazarolli, L. H., Bretanha, L. C., de Carvalho Tavares, L., da Silva Buss, Z., et al. (2014). Betulinic acid and 1,25(OH)₂ vitamin D₃ share intracellular signal transduction in glucose homeostasis in soleus muscle. *Int. J. Biochem. and Cell Biol.* 48, 18–27. doi:10.1016/j.biocel.2013.11.020
- Castro, A. J. G., Cazarolli, L. H., Bretanha, L. C., Sulis, P. M., Padilla, D. P. R., Novoa, D. M. A., et al. (2018). The potent insulin secretagogue effect of betulinic acid is mediated by potassium and chloride channels. *Archives Biochem. Biophysics* 648, 20–6. doi:10.1016/j.abb.2018.04.015
- Cercato, C., and Fonseca, F. (2019). Cardiovascular risk and obesity. *Diabetology and metabolic syndrome* 11, 74–15. doi:10.1186/s13098-019-0468-0
- Chalasani, N., Younossi, Z., Lavine, J. E., Diehl, A. M., Brunt, E. M., Cusi, K., et al. (2012). The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. *Hepatology* 55 (6), 2005–23. doi:10.1002/hep.25762
- Chambless, L. B., Parker, S. L., Hassam-Malani, L., McGirt, M. J., and Thompson, R. C. (2012). Type 2 diabetes mellitus and obesity are independent risk factors for poor outcome in patients with high-grade glioma. *J. neuro-oncology* 106, 383–9. doi:10.1007/s11060-011-0676-4
- Chan, C. B., Saleh, M. C., Koshkin, V., and Wheeler, M. B. (2004). Uncoupling protein 2 and islet function. *Diabetes* 53 (Suppl. 1_1), S136–S142. doi:10.2337/diabetes.53.2007.s136
- Chen, D.-C., Chung, Y.-F., Yeh, Y.-T., Chung, H.-C., Kuo, F.-C., Fu, O.-Y., et al. (2006). Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett.* 237 (1), 109–14. doi:10.1016/j.canlet.2005.05.047
- Chen, H., Zhang, Z.-W., Guo, Y., Wang, Y., Liu, Y., Luo, N., et al. (2012). The proliferative role of insulin and the mechanism underlying this action in human breast cancer cell line MCF-7. *J. BUON* 17 (4), 658–62. Available online at: <https://jbuon.com/archive/17-4-658.pdf>.
- Chen, Y., Wu, X., Liu, C., and Zhou, Y. (2020). Betulinic acid triggers apoptosis and inhibits migration and invasion of gastric cancer cells by impairing EMT progress. *Cell Biochem. Funct.* 38 (6), 702–9. doi:10.1002/cbf.3537
- Chen, Y., Huang, R., Mai, Z., Chen, H., Zhang, J., Zhao, L., et al. (2023). Association between systemic immune-inflammatory index and diabetes mellitus: mediation analysis involving obesity indicators in the NHANES. *Front. Public Health* 11, 1331159. doi:10.3389/fpubh.2023.1331159
- Chintharlapalli, S., Papineni, S., Ramaiah, S. K., and Safe, S. (2007). Betulinic acid inhibits prostate cancer growth through inhibition of specificity protein transcription factors. *Cancer Res.* 67 (6), 2816–23. doi:10.1158/0008-5472.CAN-06-3735
- Chintharlapalli, S., Papineni, S., Lei, P., Pathi, S., and Safe, S. (2011). Betulinic acid inhibits Colon cancer cell and tumor growth and induces proteasome-dependent and-independent downregulation of specificity proteins (sp) transcription factors. *BMC cancer* 11, 371–12. doi:10.1186/1471-2407-11-371
- Chiu, C.-F., Chang, H.-Y., Huang, C.-Y., Mau, C.-Z., Kuo, T.-T., Lee, H.-C., et al. (2021). Betulinic acid affects the energy-related proteomic profiling in pancreatic ductal adenocarcinoma cells. *Molecules* 26 (9), 2482. doi:10.3390/molecules26092482
- Choi, J.-H., Jeong, T.-S., Kim, D.-Y., Kim, Y.-M., Na, H.-J., Nam, K.-H., et al. (2003). Hematein inhibits atherosclerosis by inhibition of reactive oxygen generation and NF-kappaB-dependent inflammatory mediators in hyperlipidemic mice. *J. Cardiovasc. Pharmacol.* 42 (2), 287–95. doi:10.1097/00005344-200308000-00019
- Collaboration, E. R. F., Wormser, D., Kaptoge, S., Di Angelantonio, E., Wood, A. M., Pennells, L., et al. (2011). Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet* 377 (9771), 1085–95. doi:10.1016/S0140-6736(11)60105-0
- Cordeiro, A., Costa, R., Andrade, N., Silva, C., Canabrava, N., Pena, M. J., et al. (2020). Does adipose tissue inflammation drive the development of non-alcoholic fatty liver disease in obesity? *Clin. Res. Hepatol. Gastroenterol.* 44 (4), 394–402. doi:10.1016/j.clinre.2019.10.001
- Coricovac, D., Dehelean, C. A., Pinzaru, I., Miac, A., Aburel, O. M., Macaso, I., et al. (2021). Assessment of betulinic acid cytotoxicity and mitochondrial metabolism impairment in a human melanoma cell line. *Int. J. Mol. Sci.* 22 (9), 4870. doi:10.3390/ijms22094870
- Crosbie, E. J., Zwahlen, M., Kitchener, H. C., Egger, M., and Renehan, A. G. (2010). Body mass index, hormone replacement therapy, and endometrial cancer risk: a meta-analysis. *Cancer Epidemiol. Biomarkers and Prev.* 19 (12), 3119–30. doi:10.1158/1055-9965.EPI-10-0832
- Csuk, R. (2014). Betulinic acid and its derivatives: a patent review (2008–2013). *Expert Opin. Ther. Pat.* 24 (8), 913–23. doi:10.1517/13543776.2014.927441
- Csuk, R., Barthel, A., Schwarz, S., Kommer, H., and Paschke, R. (2010). Synthesis and biological evaluation of antitumor-active γ -butyrolactone substituted betulin derivatives. *Bioorg. and Med. Chem.* 18 (7), 2549–58. doi:10.1016/j.bmc.2010.02.042
- De Mello Del Grande, L., Herbella, F. A. M., Katayama, R. C., Lima, W. G., and Patti, M. G. (2020). Transdiaphragmatic pressure gradient (TPG) has a central role in the pathophysiology of gastroesophageal reflux disease (GERD) in the Obese and it correlates with abdominal circumference but not with body mass index (BMI). *Obes. Surg.* 30, 1424–8. doi:10.1007/s11695-019-04345-x
- Day, C. (2002). Pathogenesis of steatohepatitis. *Best Pract. and Res. Clin. gastroenterology* 16 (5), 663–78. doi:10.1053/bega.2002.0333
- de Melo, C. L., Queiroz, M. G. R., Arruda Filho, A. C. V., Rodrigues, A. M., de Sousa, D. F., Almeida, J. G. L., et al. (2009). Betulinic acid, a natural pentacyclic triterpenoid, prevents abdominal fat accumulation in mice fed a high-fat diet. *J. Agric. food Chem.* 57 (19), 8776–81. doi:10.1021/jf900768w
- Delli Bovi, A. P., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., and Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Front. Med.* 8, 595371. doi:10.3389/fmed.2021.595371

- Deng, G., Ma, C., Zhao, H., Zhang, S., Liu, J., Liu, F., et al. (2019). Anti-edema and antioxidant combination therapy for ischemic stroke via glyburide-loaded betulinic acid nanoparticles. *Theranostics* 9 (23), 6991–7002. doi:10.7150/thno.35791
- DeNicola, G. M., Karreth, F. A., Humpton, T. J., Gopinathan, A., Wei, C., Frese, K., et al. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475 (7354), 106–9. doi:10.1038/nature10189
- Dobbins, M., Decorby, K., and Choi, B. C. (2013). The association between obesity and cancer risk: a meta-analysis of observational studies from 1985 to 2011. *Int. Sch. Res. Notices* 2013 (1), 680536. doi:10.5402/2013/680536
- Du Plessis, J., van Pelt, J., Korf, H., Mathieu, C., Van Der Schueren, B., Lannoo, M., et al. (2015). Association of adipose tissue inflammation with histologic severity of nonalcoholic fatty liver disease. *Gastroenterology* 149 (3), 635–648. doi:10.1053/j.gastro.2015.05.044
- El-Serag, H. B., Tran, T., Richardson, P., and Ergun, G. (2006). Anthropometric correlates of intragastric pressure. *Scand. J. Gastroenterology* 41 (8), 887–91. doi:10.1080/00365520500535402
- Elshourbagy, N. A., Meyers, H. V., and Abdel-Meguid, S. S. (2014). Cholesterol: the good, the bad, and the ugly-therapeutic targets for the treatment of dyslipidemia. *Med. Princ. Pract.* 23 (2), 99–111. doi:10.1159/000356856
- Esievo, K. A. N., Esievo, L. O., Sani, D., Esievo, K. O., Esievo, E. M., Balogun, E., et al. (2023). Acute and delayed oral toxicity studies and observations on pregnancy, gestation and reproductive performance of wistar rats administered limit dose of purified extract from stem bark of anti-diabetic *Anogeissus leiocarpus* (african birch Tree). *J. Drug Deliv. Ther.* 13 (10), 11–27. doi:10.22270/jddt.v13i10.5965
- Esievo, L. O., Esievo, M. E., and Esievo, K. A. N. (2024). The medicinal properties of *Anogeissus leiocarpus* (African birch tree) and the development of a drug for diabetes mellitus and its complications: a review. *Univers. Libr. Med. Health Sci.* 2 (4). doi:10.70315/uloap.ulmhs.2024.0204001
- Esser, N., Legrand-Poels, S., Piette, J., Scheen, A. J., and Paquot, N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res. Clin. Pract.* 105 (2), 141–50. doi:10.1016/j.diabres.2014.04.006
- Federation, I. D. (2025). *IDF diabetes atlas*. 11th ed.
- Felber, J.-P., Ferrannini, E., Golay, A., Meyer, H. U., Theibaud, D., Curchod, B., et al. (1987). Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. *Diabetes* 36 (11), 1341–50. doi:10.2337/diab.36.11.1341
- Fernandes, S., Vieira, M., Prudêncio, C., and Ferraz, R. (2024). Betulinic acid for glioblastoma treatment: reality, challenges and perspectives. *Int. J. Mol. Sci.* 25 (4), 2108. doi:10.3390/ijms25042108
- Fortner, R. T., Katzke, V., Kühn, T., and Kaaks, R. (2016). Obesity and breast cancer. *Obes. Cancer* 208, 43–65. doi:10.1007/978-3-319-42542-9_3
- Fossati, G., Ricevuti, G., Edwards, S. W., Walker, C., Dalton, A., and Rossi, M. L. (1999). Neutrophil infiltration into human gliomas. *Acta neuropathol.* 98, 349–54. doi:10.1007/s004010051093
- Friedenreich, C. M., Ryder-Burbidge, C., and McNeil, J. (2021). Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. *Mol. Oncol.* 15 (3), 790–800. doi:10.1002/1878-0261.12772
- Fulda, S. (2008). Betulinic acid for cancer treatment and prevention. *Int. J. Mol. Sci.* 9 (6), 1096–107. doi:10.3390/ijms9061096
- Fulda, S., and Debatin, K. M. (2000). Betulinic acid induces apoptosis through a direct effect on mitochondria in neuroectodermal tumors. *Med. Pediatr. Oncol.* 35 (6), 616–618. doi:10.1002/1096-911X(20001201)35:6<616::AID-MPO27>3.0.CO;2-N
- Fulda, S., Friesen, C., Los, M., Scaffidi, C., Mier, W., Benedict, M., et al. (1997). Betulinic acid triggers CD95 (APO-1/Fas)-and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. *Cancer Res.* 57 (21), 4956–64. Available online at: <https://pubmed.ncbi.nlm.nih.gov/9354463/>.
- Fulda, S., Jeremias, I., Steiner, H. H., Pietsch, T., and Debatin, K. M. (1999). Betulinic acid: a new cytotoxic agent against malignant brain-tumor cells. *Int. J. Cancer* 82 (3), 435–41. doi:10.1002/(sici)1097-0215(19990730)82:3<435::aid-ijc18>3.0.co;2-1
- Gonzalez-Perez, R. R., Xu, Y., Guo, S., Watters, A., Zhou, W., and Leibovich, S. J. (2010). Leptin upregulates VEGF in breast cancer via canonical and non-canonical signalling pathways and NFKappaB/HIF-1alpha activation. *Cell. Signal.* 22 (9), 1350–62. doi:10.1016/j.cellsig.2010.05.003
- Gao, J., Lin, X., He, Y., Fu, Y., Wu, Y., Liao, J., et al. (2019). The comparison of different obesity indexes and the risk of lung cancer: a meta-analysis of prospective cohort studies. *Nutr. Cancer* 71 (6), 908–21. doi:10.1080/01635581.2019.1595037
- Gallagher, E. J., and LeRoith, D. (2010). The proliferating role of insulin and insulin-like growth factors in cancer. *Trends Endocrinol. and Metabolism* 21 (10), 610–8. doi:10.1016/j.tem.2010.06.007
- Garofalo, C., Koda, M., Cascio, S., Sulkowska, M., Kanczuga-Koda, L., Golaszewska, J., et al. (2006). Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. *Clin. Cancer Res.* 12 (5), 1447–53. doi:10.1158/1078-0432.CCR-05-1913
- Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M., DeFronzo, R., and San Antonio metabolism study (2004). Beta-cell dysfunction and glucose intolerance: results from the san antonio metabolism (SAM) study. *Diabetologia* 47, 31–9. doi:10.1007/s00125-003-1263-9
- Gaudet, M. M., Press, M. F., Haile, R. W., Lynch, C. F., Glaser, S. L., Schildkraut, J., et al. (2011). Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger. *Breast cancer Res. Treat.* 130, 587–97. doi:10.1007/s10549-011-1616-x
- Genkinger, J. M., Spiegelman, D., Anderson, K. E., Bernstein, L., Van Den Brandt, P. A., Calle, E. E., et al. (2011). A pooled analysis of 14 cohort studies of anthropometric factors and pancreatic cancer risk. *Int. J. Cancer* 129 (7), 1708–17. doi:10.1002/ijc.25794
- Gheorgheos, D., Jung, M., Ören, B., Schmid, T., Dehelean, C., Muntean, D., et al. (2013). Betulinic acid suppresses NGAL-Induced epithelial-to-mesenchymal transition in melanoma. *Biol. Chem.* 394 (6), 773–81. doi:10.1515/hsz-2013-0106
- Gielecińska, A., Kciuk, M., Mujwar, S., Celik, I., Kolat, D., Kałuzińska-Kolat, Ż., et al. (2023). Substances of natural origin in medicine: plants vs. cancer. *Cells* 12 (7), 986. doi:10.3390/cells12070986
- Giorgio, V., Miele, L., Principessa, L., Ferretti, F., Villa, M. P., Negro, V., et al. (2014). Intestinal permeability is increased in children with non-alcoholic fatty liver disease, and correlates with liver disease severity. *Dig. Liver Dis.* 46 (6), 556–60. doi:10.1016/j.dld.2014.02.010
- Godugu, C., Patel, A. R., Doddapaneni, R., Somagoni, J., and Singh, M. (2014). Approaches to improve the oral bioavailability and effects of novel anticancer drugs berberine and betulinic acid. *PLoS one* 9 (3), e89919. doi:10.1371/journal.pone.0089919
- Golay, A., Felber, J., Meyer, H., Curchod, B., Maeder, E., and Jéquier, E. (1984). Study on lipid metabolism in obesity diabetes. *Metabolism* 33 (2), 111–6. doi:10.1016/0026-0495(84)90121-5
- Gómez-Ambrosi, J., Salvador, J., Páramo, J. A., Orbe, J., de Irala, J., Diez-Caballero, A., et al. (2002). Involvement of leptin in the association between percentage of body fat and cardiovascular risk factors. *Clin. Biochem.* 35 (4), 315–20. doi:10.1016/s0009-9120(02)00320-x
- Gonçalves, I. O., Passos, E., Rocha-Rodrigues, S., Diogo, C. V., Torrella, J. R., Rizo, D., et al. (2014). Physical exercise prevents and mitigates non-alcoholic steatohepatitis-induced liver mitochondrial structural and bioenergetics impairments. *Mitochondrion* 15, 40–51. doi:10.1016/j.mito.2014.03.012
- Gu, M., Zhao, P., Zhang, S., Fan, S., Yang, L., Tong, Q., et al. (2019). Betulinic acid alleviates endoplasmic reticulum stress-mediated nonalcoholic fatty liver disease through activation of farnesoid X receptors in mice. *Br. J. Pharmacol.* 176 (7), 847–63. doi:10.1111/bph.14570
- Guilherme, A., Virbasius, J. V., Puri, V., and Czech, M. P. (2008). Adipocyte dysfunction linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* 9 (5), 367–77. doi:10.1038/nrm2391
- Guo, Y., Zhu, H., Weng, M., Wang, C., and Sun, L. (2020). Chemopreventive effect of betulinic acid via mTOR-Caspases/Bcl2/Bax apoptotic signaling in pancreatic cancer. *BMC Complementary Med. Ther.* 20, 178–11. doi:10.1186/s12906-020-02976-7
- Hägerstrand, D., Lindh, M. B., Pena, C., Garcia-Echeverria, C., Nister, M., Hofmann, F., et al. (2010). PI3K/PTEN/Akt pathway status affects the sensitivity of high-grade glioma cell cultures to the insulin-like growth factor-1 receptor inhibitor NVP-AEW541. *Neuro-oncology* 12 (9), 967–75. doi:10.1093/neonc/nq029
- Haque, R., Van Den Eeden, S. K., Wallner, L. P., Richert-Boe, K., Kallakury, B., Wang, R., et al. (2014). Association of body mass index and prostate cancer mortality. *Obes. Res. and Clin. Pract.* 8 (4), e374–e381. doi:10.1016/j.orcp.2013.06.002
- Hardy, T., Oakley, F., Anstee, Q. M., and Day, C. P. (2016). Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. *Annu. Rev. pathology Mech. Dis.* 11 (1), 451–96. doi:10.1146/annurev-pathol-012615-044224
- Harvey, S. V., Wentzensen, N., Bertrand, K., Black, A., Brinton, L. A., Chen, C., et al. (2023). Associations of life course obesity with endometrial cancer in the epidemiology of endometrial cancer consortium (E2C2). *Int. J. Epidemiol.* 52 (4), 1086–99. doi:10.1093/ije/dyad046
- Hauner, H. (2016). “Obesity and diabetes.” in *Textbook of Diabetes 2016*, 215–28.
- Hirosumi, J., Tuncman, G., Chang, L., Görgün, C. Z., Uysal, K. T., Maeda, K., et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature* 420 (6913), 333–6. doi:10.1038/nature01137
- Hoyo, C., Cook, M. B., Kamangar, F., Freedman, N. D., Whiteman, D. C., Bernstein, L., et al. (2012). Body mass index in relation to oesophageal and oesophagogastric junction adenocarcinomas: a pooled analysis from the international BEACON consortium. *Int. J. Epidemiol.* 41 (6), 1706–18. doi:10.1093/ije/dys176
- Hsu, T. I., Wang, M. C., Chen, S. Y., Huang, S. T., Yeh, Y. M., Su, W. C., et al. (2012). Betulinic acid decreases specificity protein 1 (Sp1) level via increasing the sumoylation of sp1 to inhibit lung cancer growth. *Mol. Pharmacol.* 82 (6), 1115–28. doi:10.1124/mol.112.078485
- Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M., Ferrannini, E., Nauck, M., et al. (2015). Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American diabetes association and the european association for the Study of Diabetes. *Diabetes care* 38 (1), 140–9. doi:10.2337/dc14-2441
- Ionescu, V. A., Gheorghe, G., Bacalbasa, N., Chiotoroiu, A. L., and Diaconu, C. (2023). Colorectal cancer: from risk factors to oncogenesis. *Med. Kaunas.* 59 (9), 1646. doi:10.3390/medicina59091646

- Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., et al. (2003). Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature* 422 (6928), 173–6. doi:10.1038/nature01478
- Iyengar, N. M., Hudis, C. A., and Dannenberg, A. J. (2013). Obesity and inflammation: new insights into breast cancer development and progression. *Am. Soc. Clin. Oncol. Educ. Book* 33 (1), 46–51. doi:10.14694/EdBook_AM.2013.33.46
- Jäger, S., Trojan, H., Kopp, T., Laszczyk, M. N., and Scheffler, A. (2009). Pentacyclic triterpene distribution in various plants—rich sources for a new group of multi-potent plant extracts. *Molecules* 14 (6), 2016–31. doi:10.3390/molecules14062016
- Jarrar, M., Baranova, A., Collantes, R., Ranard, B., Stepanova, M., Bennett, C., et al. (2008). Adipokines and cytokines in non-alcoholic fatty liver disease. *Alimentary Pharmacol. and Ther.* 27 (5), 412–21. doi:10.1111/j.1365-2036.2007.03586.x
- Jiang, W., Li, X., Dong, S., and Zhou, W. (2021). Betulinic acid in the treatment of tumour diseases: application and research progress. *Biomed. and Pharmacother.* 142, 111990. doi:10.1016/j.biopha.2021.111990
- Jin, S. W., Choi, C. Y., Hwang, Y. P., Kim, H. G., Kim, S. J., Chung, Y. C., et al. (2016). Betulinic acid increases eNOS phosphorylation and NO synthesis via the calcium-signaling pathway. *J. Agric. food Chem.* 64 (4), 785–91. doi:10.1021/acs.jafc.5b05416
- Jung, K. W., Talley, N. J., Romero, Y., Katzka, D. A., Schleck, C. D., Zinsmeister, A. R., et al. (2011). Epidemiology and natural history of intestinal metaplasia of the gastroesophageal junction and Barrett's esophagus: a population-based study. *Official J. Am. Coll. Gastroenterology| ACG.* 106 (8), 1447–55. doi:10.1038/ajg.2011.130
- Kahn, S. E. (2001). Clinical review 135: the importance of beta-cell failure in the development and progression of type 2 diabetes. *J. Clin. Endocrinol. and Metabolism* 86 (9), 4047–58. doi:10.1210/jcem.86.9.7713
- Kahn, S. E., Hull, R. L., and Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444 (7121), 840–6. doi:10.1038/nature05482
- Karczewski, J., Śledzińska, E., Batur, A., Jończyk, I., Maleszko, A., Samborski, P., et al. (2018). Obesity and inflammation. *Eur. cytokine Netw.* 29, 83–94. doi:10.1684/ecn.2018.0415
- Karlsson, C. L., Önerfält, J., Xu, J., Molin, G., Ahnér, S., and Thorgren-Jerneck, K. (2012). The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* 20 (11), 2257–61. doi:10.1038/oby.2012.110
- Karna, E., and Palka, J. A. (2009). Mechanism of betulinic acid inhibition of collagen biosynthesis in human endometrial adenocarcinoma cells. *Neoplasma* 56 (4), 361–6. doi:10.4149/neo_2009_04_361
- Karna, E., Szoka, L., and Palka, J. A. (2010). Betulinic acid inhibits the expression of hypoxia-inducible factor 1 α and vascular endothelial growth factor in human endometrial adenocarcinoma cells. *Mol. Cell. Biochem.* 340, 15–20. doi:10.1007/s11010-010-0395-8
- Karra, P., Winn, M., Pauleck, S., Bulsiewicz-Jacobsen, A., Peterson, L., Coletta, A., et al. (2022). Metabolic dysfunction and obesity-related cancer: beyond obesity and metabolic syndrome. *Obesity* 30 (7), 1323–34. doi:10.1002/oby.23444
- Kaur, R., Bhardwaj, A., and Gupta, S. (2023). Cancer treatment therapies: traditional to modern approaches to combat cancers. *Mol. Biol. Rep.* 50 (11), 9663–76. doi:10.1007/s11033-023-08809-3
- Keane, K. N., Cruzat, V. F., Carlessi, R., de, B., Jr, and Newsholme, P. (2015). Molecular events linking oxidative stress and inflammation to insulin resistance and β -cell dysfunction. *Oxidative Med. Cell. Longev.* 2015 (1), 181643. doi:10.1155/2015/181643
- Kenchiah, S., Evans, J. C., Levy, D., Wilson, P. W., Benjamin, E. J., Larson, M. G., et al. (2002). Obesity and the risk of heart failure. *N. Engl. J. Med.* 347 (5), 305–13. doi:10.1056/NEJMoa020245
- Kessler, J. H., Mullauer, F. B., de Roo, G. M., and Medema, J. P. (2007). Broad *in vitro* efficacy of plant-derived betulinic acid against cell lines derived from the most prevalent human cancer types. *Cancer Lett.* 251 (1), 132–45. doi:10.1016/j.canlet.2006.11.003
- Key, T., and Pike, M. (1988). The dose-effect relationship between unopposed oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br. J. cancer* 57 (2), 205–12. doi:10.1038/bjc.1988.44
- Killeen, S., Wang, J., Andrews, E., and Redmond, H. (2009). Bacterial endotoxin enhances colorectal cancer cell adhesion and invasion through TLR-4 and NF-kappaB-dependent activation of the urokinase plasminogen activator system. *Br. J. cancer* 100 (10), 1589–602. doi:10.1038/sj.bjc.6604942
- Killinger, B., Shah, M., and Moszczynska, A. (2014). Co-administration of betulinic acid and methamphetamine causes toxicity to dopaminergic and serotonergic nerve terminals in the striatum of late adolescent rats. *J. Neurochem.* 128 (5), 764–75. doi:10.1111/jnc.12496
- Kim, S. J., Quan, H. Y., Jeong, K. J., Kim, D. Y., Kim, G. W., Jo, H. K., et al. (2014a). Beneficial effect of betulinic acid on hyperglycemia via suppression of hepatic glucose production. *J. Agric. food Chem.* 62 (2), 434–42. doi:10.1021/jf4030739
- Kim, H.-I., Quan, F.-S., Kim, J.-E., Lee, N.-R., Kim, H. J., Jo, S. J., et al. (2014b). Inhibition of estrogen signaling through depletion of estrogen receptor alpha by ursolic acid and betulinic acid from *Prunella vulgaris* var. *ilicina*. *Biochem. biophysical Res. Commun.* 451 (2), 282–7. doi:10.1016/j.bbrc.2014.07.115
- Kim, S. H., Després, J.-P., and Koh, K. K. (2016). Obesity and cardiovascular disease: friend or foe? *Eur. heart J.* 37 (48), 3560–8. doi:10.1093/eurheartj/ehv509
- Kim, K.-D., Jung, H.-Y., Ryu, H., Kim, B., Jeon, J., Yoo, H., et al. (2019). Betulinic acid inhibits high-fat diet-induced obesity and improves energy balance by activating AMPK. *Metabolism Cardiovasc. Dis.* 29 (4), 409–20. doi:10.1016/j.numecd.2018.12.001
- Kiraz, Y., Adan, A., Kartal Yandim, M., and Baran, Y. (2016). Major apoptotic mechanisms and genes involved in apoptosis. *Tumor Biol.* 37, 8471–86. doi:10.1007/s13277-016-5035-9
- Koshy, M., Villano, J. L., Dolecek, T. A., Howard, A., Mahmood, U., Chmura, S. J., et al. (2012). Improved survival time trends for glioblastoma using the SEER 17 population-based registries. *J. neuro-oncology* 107, 207–12. doi:10.1007/s11060-011-0738-7
- Krauss, R. M., Winston, M., Fletcher, B. J., and Grundy, S. M. (1998). Obesity: impact of cardiovascular disease. *Circulation* 98 (14), 1472–6. doi:10.1161/01.cir.98.14.1472
- Kumar, S., Kumar, V., and Prakash, O. (2013). Enzymes inhibition and antidiabetic effect of isolated constituents from *Dillenia indica*. *BioMed Res. Int.* 2013 (1), 382063. doi:10.1155/2013/382063
- Kumar, P., Bhaduria, A. S., Singh, A. K., and Saha, S. (2018). Betulinic acid as apoptosis activator: molecular mechanisms, mathematical modeling and chemical modifications. *Life Sci.* 209, 24–33. doi:10.1016/j.lfs.2018.07.056
- Kuzminova, N. V., Gribenyuk, O. V., Osovska, N. Y., and Knyazkova, I. I. (2016). Arterial hypertension, obesity and non-alcoholic fatty liver disease: is there any connection? *Arterial. Hypertension* 20 (4), 216–27. doi:10.5603/ah.2016.0025
- Landecho, M. F., Tuero, C., Valentí, V., Bilbao, I., de la Higuera, M., and Frühbeck, G. (2019). Relevance of leptin and other adipokines in obesity-associated cardiovascular risk. *Nutrients* 11 (11), 2664. doi:10.3390/nu11112664
- Lavie, C. J., McAuley, P. A., Church, T. S., Milani, R. V., and Blair, S. N. (2014). Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. *J. Am. Coll. Cardiol.* 63 (14), 1345–54. doi:10.1016/j.jacc.2014.01.022
- Lee, S. R., Kwon, K. S., Kim, S. R., and Rhee, S. G. (1998). Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J. Biol. Chem.* 273 (25), 15366–72. doi:10.1074/jbc.273.25.15366
- Lee, D., Lee, S. R., Kang, K. S., Ko, Y., Pang, C., Yamabe, N., et al. (2019). Betulinic acid suppresses ovarian cancer cell proliferation through induction of apoptosis. *Biomolecules* 9 (7), 257. doi:10.3390/biom9070257
- Leitzmann, M. F., Stein, M. J., Baurecht, H., and Freisling, H. (2025). Excess adiposity and cancer: evaluating a preclinical-clinical obesity framework for risk stratification. *eClinicalMedicine* 83, 103247. doi:10.1016/j.eclinm.2025.103247
- Lemstra, M., Bird, Y., Nwankwo, C., Rogers, M., and Moraros, J. (2016). Weight loss intervention adherence and factors promoting adherence: a meta-analysis. *Patient Prefer Adherence* 10, 1547–59. doi:10.2147/PPA.S103649
- Li, Y., Wang, Y., Gao, L., Tan, Y., Cai, J., Ye, Z., et al. (2022). Betulinic acid self-assembled nanoparticles for effective treatment of glioblastoma. *J. nanobiotechnology* 20 (1), 39. doi:10.1186/s12951-022-01238-7
- Liao, L., Liu, C., Xie, X., and Zhou, J. (2020). Betulinic acid induces apoptosis and impairs migration and invasion in a mouse model of ovarian cancer. *J. Food Biochem.* 44 (7), e13278. doi:10.1111/jfbc.13278
- Liu, W. K., Ho, J. C., Cheung, F. W., Liu, B. P., Ye, W. C., and Che, C. T. (2004). Apoptotic activity of betulinic acid derivatives on murine melanoma B16 cell line. *Eur. J. Pharmacol.* 498 (1-3), 71–8. doi:10.1016/j.ejphar.2004.07.103
- Liu, X., Jutooru, I., Lei, P., Kim, K., Lee, S.-o., Brents, L. K., et al. (2012). Betulinic acid targets YY1 and ErbB2 through cannabinoid receptor-dependent disruption of microRNA-27a: ZBTB10 in breast cancer. *Mol. cancer Ther.* 11 (7), 1421–31. doi:10.1158/1535-7163.MCT-12-0026
- Liu, W.-T., Jing, Y.-Y., Yan, F., Han, Z.-P., Lai, F.-B., Zeng, J.-X., et al. (2017). LPS-induced CXCR4-dependent migratory properties and a mesenchymal-like phenotype of colorectal cancer cells. *Cell Adhesion and Migr.* 11 (1), 13–23. doi:10.1080/19336918.2015.1134404
- Lo, W.-L., Hsu, T.-I., Yang, W.-B., Kao, T.-J., Wu, M.-H., Huang, Y.-N., et al. (2020). Betulinic acid-mediated tuning of PERK/CHOP signaling by Sp1 inhibition as a novel therapeutic strategy for glioblastoma. *Cancers* 12 (4), 981. doi:10.3390/cancers12040981
- Lois, K., and Kumar, S. (2009). Obesity and diabetes. *Endocrinol. Nutr.* 56, 38–42. doi:10.1016/s1575-0922(09)73516-8
- Lou, H., Li, H., Zhang, S., Lu, H., and Chen, Q. (2021). A review on preparation of betulinic acid and its biological activities. *Molecules* 26 (18), 5583. doi:10.3390/molecules26185583
- Luo, R., Fang, D., Chu, P., Wu, H., Zhang, Z., and Tang, Z. (2016). Multiple molecular targets in breast cancer therapy by betulinic acid. *Biomed. and Pharmacother.* 84, 1321–30. doi:10.1016/j.biopha.2016.10.018
- Machado, M., Marques-Vidal, P., and Cortez-Pinto, H. (2006). Hepatic histology in obese patients undergoing bariatric surgery. *J. hepatology* 45 (4), 600–6. doi:10.1016/j.jhep.2006.06.013

- Malaczewska, J., Kaczorek-Lukowska, E., and Kazań, B. (2021). High cytotoxicity of betulin towards fish and murine fibroblasts: is betulin safe for nonneoplastic cells? *BMC Veterinary Res.* 17 (1), 198. doi:10.1186/s12917-021-02905-x
- Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C., and Del Cañizo-Gómez, F. J. (2016). Update on the treatment of type 2 diabetes mellitus. *World J. Diabetes* 7 (17), 354–95. doi:10.4239/wjcd.v7.i17.354
- Marino, P., Mininni, M., Deiana, G., Marino, G., Divella, R., Bochicchio, I., et al. (2024). Healthy lifestyle and cancer risk: modifiable risk factors to prevent cancer. *Nutrients* 16 (6), 800. doi:10.3390/nu16060800
- Mcgarry, J. D., Mannaerts, G. P., and Foster, D. W. (1977). A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. *J. Clin. Investigation* 60 (1), 265–70. doi:10.1172/JCI108764
- Melzig, M. F., and Bormann, H. (1998). Betulinic acid inhibits aminopeptidase N activity. *Planta medica*. 64 (07), 655–7. doi:10.1055/s-2006-957542
- Meshkani, R., and Adeli, K. (2009). Hepatic insulin resistance, metabolic syndrome and cardiovascular disease. *Clin. Biochem.* 42 (13–14), 1331–46. doi:10.1016/j.clinbiochem.2009.05.018
- Miele, L., Valenza, V., La Torre, G., Montalto, M., Cammarota, G., Ricci, R., et al. (2009). Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 49 (6), 1877–87. doi:10.1002/hep.22848
- Mobley, J. A., and Brueggemeier, R. W. (2004). Estrogen receptor-mediated regulation of oxidative stress and DNA damage in breast cancer. *Carcinogenesis* 25 (1), 3–9. doi:10.1093/carcin/bgg175
- Modesitt, S. C., Tian, C., Kryscio, R., Thigpen, J. T., Randall, M. E., Gallion, H. H., et al. (2007). Impact of body mass index on treatment outcomes in endometrial cancer patients receiving doxorubicin and cisplatin: a Gynecologic Oncology Group study. *Gynecol. Oncol.* 105 (1), 59–65. doi:10.1016/j.ygyno.2006.10.045
- Mu, Q., Wang, H., Tong, L., Fang, Q., Xiang, M., Han, L., et al. (2020). Betulinic acid improves nonalcoholic fatty liver disease through YY1/FAS signaling pathway. *Faseb J.* 34 (9), 13033–48. doi:10.1096/fj.202000546R
- Mukherjee, D., Kumar, N. S., Khatua, T., and Mukherjee, P. K. (2010). Rapid validated HPTLC method for estimation of betulinic acid in *Nelumbo nucifera* (Nymphaeaceae) rhizome extract. *Phytochem. Anal.* 21 (6), 556–60. doi:10.1002/pca.1232
- Mullauer, F. B., Kessler, J. H., and Medema, J. P. (2010). Betulinic acid, a natural compound with potent anticancer effects. *Anticancer Drugs* 21 (3), 215–27. doi:10.1097/CAD.0b013e3283357c62
- Murat, A., Migliavacca, E., Hussain, S. F., Heimberger, A. B., Desbaillets, I., Hamou, M.-F., et al. (2009). Modulation of angiogenic and inflammatory response in glioblastoma by hypoxia. *PLoS one* 4 (6), e5947. doi:10.1371/journal.pone.0005947
- Murphy, S. H., Suzuki, K., Downes, M., Welch, G. L., De Jesus, P., Miraglia, L. J., et al. (2011). Tumor suppressor protein (p)53, is a regulator of NF-kappaB repression by the glucocorticoid receptor. *Proc. Natl. Acad. Sci.* 108 (41), 17117–22. doi:10.1073/pnas.1114420108
- Muruzábal, F. J., Frühbeck, G., Gómez-Ambrosi, J., Archanco, M., and MaA, B. (2002). Immunocytochemical detection of leptin in non-mammalian vertebrate stomach. *General Comp. Endocrinol.* 128 (2), 149–52. doi:10.1016/s0016-6480(02)00072-2
- Nacht, M., Kwanten, W. J., Thissen, J.-P., De Beeck, B. O., Van Gaal, L., Vonghia, L., et al. (2021). Muscle fat content is strongly associated with NASH: a longitudinal study in patients with morbid obesity. *J. hepatology* 75 (2), 292–301. doi:10.1016/j.jhep.2021.02.037
- Nakamura, K., Fuster, J. J., and Walsh, K. (2014). Adipokines: a link between obesity and cardiovascular disease. *J. Cardiol.* 63 (4), 250–9. doi:10.1016/j.jcc.2013.11.006
- Nguyen, T. T., Ung, T. T., Kim, N. H., and Do Jung, Y. (2018). Role of bile acids in colon carcinogenesis. *World J. Clin. Cases* 6 (13), 577–588. doi:10.12998/wjcc.v6.i13.577
- Nisar, S., Masoodi, T., Prabhu, K. S., Kuttikrishnan, S., Zarif, L., Khatoun, S., et al. (2022). Natural products as chemo-radiation therapy sensitizers in cancers. *Biomed. and Pharmacother.* 154, 113610. doi:10.1016/j.biopha.2022.113610
- Noda, T., Kikugawa, T., Tanji, N., Miura, N., Asai, S., Higashiyama, S., et al. (2015). Long-term exposure to leptin enhances the growth of prostate cancer cells. *Int. J. Oncol.* 46 (4), 1535–42. doi:10.3892/ijo.2015.2845
- Ochsenkühn, T., Bayerdörffer, E., Meining, A., Schinkel, M., Thiede, C., Nüssler, V., et al. (1999). Colonic mucosal proliferation is related to serum deoxycholic acid levels. *Cancer Interdiscip. Int. J. Am. Cancer Soc.* 85 (8), 1664–9.
- Olivas, A., and Price, R. S. (2021). Obesity, inflammation, and advanced prostate cancer. *Nutr. Cancer* 73 (11–12), 2232–48. doi:10.1080/01635581.2020.1856889
- Oliveira-Costa, J. F., Meira, C. S., Neves, M. V. G., Dos Reis, BPZC, and Soares, M. B. P. (2022). Anti-inflammatory activities of betulinic acid: a review. *Front. Pharmacol.* 13, 883857. doi:10.3389/fphar.2022.883857
- Onstad, M. A., Schmandt, R. E., and Lu, K. H. (2016). Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *J. Clin. Oncol.* 34 (35), 4225–30. doi:10.1200/JCO.2016.69.4638
- Organization, W. H. (2024). Global cancer burden growing, amidst mounting need for services Lyon, France; Geneva, Switzerland. Available online at: <https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing-amidst-mounting-need-for-services>.
- Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., and Zuñiga, F. A. (2018). Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc. Diabetol.* 17, 122–14. doi:10.1186/s12933-018-0762-4
- Ortega, F. B., Lavie, C. J., and Blair, S. N. (2016). Obesity and cardiovascular disease. *Circ. Res.* 118 (11), 1752–70. doi:10.1161/CIRCRESAHA.115.306883
- Padhi, S., Nayak, A. K., and Behera, A. (2020). Type II diabetes mellitus: a review on recent drug based therapeutics. *Biomed. and Pharmacother.* 131, 110708. doi:10.1016/j.biopha.2020.110708
- Panza, S., Russo, U., Giordano, F., Leggio, A., Barone, I., Bonofiglio, D., et al. (2020). Leptin and notch signaling cooperate in sustaining glioblastoma multiforme progression. *Biomolecules* 10 (6), 886. doi:10.3390/biom10060886
- Pavlova, N., Savinova, O., Nikolaeva, S., Boreko, E., and Flekhter, O. (2003). Antiviral activity of betulin, betulonic and betulonic acids against some enveloped and non-enveloped viruses. *Fitoterapia* 74 (5), 489–92. doi:10.1016/s0367-326x(03)00123-0
- Pearson-Stuttard, J., Zhou, B., Kontis, V., Bentham, J., Gunter, M. J., and Ezzati, M. (2018). Worldwide burden of cancer attributable to diabetes and high body-mass index: a comparative risk assessment. *Lancet Diabetes and Endocrinol.* 6 (2), 95–104. doi:10.1016/S2213-8587(17)30366-2
- Peiseler, M., Schwabe, R., Hampe, J., Kubes, P., Heikenwälder, M., and Tacke, F. (2022). Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J. Hepatol.* 77 (4), 1136–60. doi:10.1016/j.jhep.2022.06.012
- Péqueux, C., Raymond-Letron, I., Blacher, S., Boudou, F., Adlanmerini, M., Fouque, M.-J., et al. (2012). Stromal estrogen receptor- α promotes tumor growth by normalizing an increased angiogenesis. *Cancer Res.* 72 (12), 3010–9. doi:10.1158/0008-5472.CAN-11-3768
- Pereira, S. S., and Alvarez-Leite, J. I. (2014). Low-grade inflammation, obesity, and diabetes. *Curr. Obes. Rep.* 3, 422–31. doi:10.1007/s13679-014-0124-9
- Perla, F. M., Prelati, M., Lavorato, M., Visicchio, D., and Anania, C. (2017). The role of lipid and lipoprotein metabolism in non-alcoholic fatty liver disease. *Children* 4 (6), 46. doi:10.3390/children4060046
- Petersen, K. F., Dufour, S., Savage, D. B., Bilz, S., Solomon, G., Yonemitsu, S., et al. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc. Natl. Acad. Sci.* 104 (31), 12587–94. doi:10.1073/pnas.0705408104
- Pezzino, S., Sofia, M., Faletta, G., Mazzone, C., Litrico, G., La Greca, G., et al. (2022). Gut-liver axis and non-alcoholic fatty liver disease: a vicious circle of dysfunctions orchestrated by the gut microbiome. *Biology* 11 (11), 1622. doi:10.3390/biology11111622
- Poitout, V., and Robertson, R. P. (2008). Glucolipotoxicity: fuel excess and β -cell dysfunction. *Endocr. Rev.* 29 (3), 351–66. doi:10.1210/er.2007-0023
- Poloz, Y., and Stambolic, V. (2015). Obesity and cancer, a case for insulin signaling. *Cell Death and Dis.* 6 (12), e2037–e. doi:10.1038/cddis.2015.381
- Polyzos, S. A., Kountouras, J., and Mantzoros, C. S. (2016). Adipose tissue, obesity and non-alcoholic fatty liver disease. *Minerva Endocrinol.* 42 (2), 92–108. doi:10.23736/S0391-1977.16.02563-3
- Polyzos, S. A., Kountouras, J., and Mantzoros, C. S. (2019). Obesity and nonalcoholic fatty liver disease: from pathophysiology to therapeutics. *Metabolism* 92, 82–97. doi:10.1016/j.metabol.2018.11.014
- Porras, D., Nistal, E., Martínez-Flórez, S., González-Gallego, J., García-Mediavilla, M. V., and Sánchez-Campos, S. (2018). Intestinal microbiota modulation in obesity-related non-alcoholic fatty liver disease. *Front. Physiol.* 9, 1813. doi:10.3389/fphys.2018.01813
- Quan, H. Y., Kim, S. J., Jo, H. K., Kim, G. W., and Chung, S. H. (2013). Betulinic acid alleviates non-alcoholic fatty liver by inhibiting SREBP1 activity via the AMPK-mTOR-SREBP signaling pathway. *Biochem. Pharmacol.* 85 (9), 1330–40. doi:10.1016/j.bcp.2013.02.007
- Quillen, D. M., Samraj, G., and Kuritzky, L. (1999). Improving management of type 2 diabetes mellitus: 2. Biguanides. *Hosp. Pract.* 34 (11), 41–4. doi:10.1080/21548331.1999.11443925
- Rabi, T., Shukla, S., and Gupta, S. (2008). Betulinic acid suppresses constitutive and TNF α -induced NF-kappaB activation and induces apoptosis in human prostate carcinoma PC-3 cells. *Mol. Carcinog. Publ. Coop. Univ. Tex. MD Anderson Cancer Cent.* 47 (12), 964–73. doi:10.1002/mc.20447
- Reid, B. J., Sanchez, C. A., Blount, P. L., and Levine, D. S. (1993). Barrett's esophagus: cell cycle abnormalities in advancing stages of neoplastic progression. *Gastroenterology* 105 (1), 119–29. doi:10.1016/0016-5085(93)90017-7
- Reiner, T., Parrondo, R., de Las Pozas, A., Palenzuela, D., and Perez-Stable, C. (2013). Betulinic acid selectively increases protein degradation and enhances prostate cancer-specific apoptosis: possible role for inhibition of deubiquitinase activity. *PLoS one* 8 (2), e56234. doi:10.1371/journal.pone.0056234
- Ríos, J. L., and Mániz, S. (2018). New pharmacological opportunities for betulinic acid. *Planta Medica*. 84 (01), 8–19. doi:10.1055/s-0043-123472

- Riya, M. P., Antu, K. A., Pal, S., Srivastava, A. K., Sharma, S., and Raghu, K. G. (2014). Nutraceutical potential of *Aerva lanata* (L.) juss. Ex schult ameliorates secondary complications in streptozotocin-induced diabetic rats. *Food and Funct.* 5 (9), 2086–95. doi:10.1039/c4fo00013g
- Robins, S. J., Lyass, A., Zachariah, J. P., Massaro, J. M., and Vasan, R. S. (2011). Insulin resistance and the relationship of a dyslipidemia to coronary heart disease: the Framingham Heart Study. *Arterioscler. Thromb. Vasc. Biol.* 31 (5), 1208–14. doi:10.1161/ATVBAHA.110.219055
- Rodríguez, A., Gómez-Ambrosi, J., Catalán, V., Fortuño, A., and Frühbeck, G. (2010). Leptin inhibits the proliferation of vascular smooth muscle cells induced by angiotensin II through nitric oxide-dependent mechanisms. *Mediat. Inflamm.* 2010 (1), 105489. doi:10.1155/2010/105489
- Rodríguez, A., Ezquerro, S., Méndez-Giménez, L., Becerril, S., and Frühbeck, G. (2015). Revisiting the adipocyte: a model for integration of cytokine signaling in the regulation of energy metabolism. *Am. J. Physiology-Endocrinology Metabolism* 309 (8), E691–E714. doi:10.1152/ajpendo.00297.2015
- Roque, D., Cruz, N., Ferreira, H. A., Reis, C. P., Matela, N., Herculano-Carvalho, M., et al. (2023). Nanoparticle-Based treatment in glioblastoma. *J. Personalized Med.* 13 (9), 1328. doi:10.3390/jpm13091328
- Ruiz-Molina, D., Mao, X., Alfonso-Triguero, P., Lorenzo, J., Bruna, J., Yuste, V. J., et al. (2022). Advances in preclinical/clinical glioblastoma treatment: can nanoparticles be of help? *Cancers* 14 (19), 4960. doi:10.3390/cancers14194960
- Ryan, A. S., Berman, D. M., Nicklas, B. J., Sinha, M., Gingerich, R. L., Meneilly, G. S., et al. (2003). Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. *Diabetes Care* 26 (8), 2383–8. doi:10.2337/diacare.26.8.2383
- Said, A., and Akhter, A. (2017). Meta-analysis of randomized controlled trials of pharmacologic agents in non-alcoholic steatohepatitis. *Ann. Hepatology* 16 (4), 538–47. doi:10.5604/01.3001.0010.0284
- Salau, V., Erukainure, O., Aljoundi, A., Akintemi, E., Elamin, G., and Odewole, O. (2024). Exploring the inhibitory action of betulinic acid on key digestive enzymes linked to diabetes *via in vitro* and computational models: approaches to anti-diabetic mechanisms. *SAR QSAR Environ. Res.* 35, 411–432. doi:10.1080/1062936X.2024.2352729
- Sanyal, A. J., Campbell-Sargent, C., Mirshahi, F., Rizzo, W. B., Contos, M. J., Sterling, R. K., et al. (2001). Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120 (5), 1183–92. doi:10.1053/gast.2001.23256
- Sarwar, R., Pierce, N., and Koppe, S. (2018). Obesity and nonalcoholic fatty liver disease: current perspectives. *Diabetes, Metabolic Syndrome Obes. Targets Ther.* 11, 533–42. doi:10.2147/DMSO.S146339
- Schlenska-Lange, A., Knüpfer, H., Lange, T. J., Kiess, W., and Knüpfer, M. (2008). Cell proliferation and migration in glioblastoma multiforme cell lines are influenced by insulin-like growth factor I *in vitro*. *Anticancer Res.* 28 (2A), 1055–60. Available online at: <https://pubmed.ncbi.nlm.nih.gov/18507054/>.
- Schlottmann, F., Dreifuss, N. H., and Patti, M. G. (2020). Obesity and esophageal cancer: GERD, Barrett's esophagus, and molecular carcinogenic pathways. *Expert Rev. Gastroenterology and Hepatology* 14 (6), 425–33. doi:10.1080/17474124.2020.1764348
- Schneider, G., Henrich, A., Greiner, G., Wolf, V., Lovas, A., Wiczorek, M., et al. (2010). Cross talk between stimulated NF-kappaB and the tumor suppressor p53. *Oncogene* 29 (19), 2795–806. doi:10.1038/ncr.2010.46
- Schühly, W., Heilmann, J., Çalis, I., and Sticher, O. (1999). New triterpenoids with antibacterial activity from *Zizyphus joazeiro*. *Planta Medica*. 65 (08), 740–3. doi:10.1055/s-1999-14054
- Selzer, E., Pimentel, E., Wacheck, V., Schlegel, W., Pehamberger, H., Jansen, B., et al. (2000). Effects of betulinic acid alone and in combination with irradiation in human melanoma cells. *J. Investigative Dermatology* 114 (5), 935–40. doi:10.1046/j.1523-1747.2000.00972.x
- Seyfried, T. N., Flores, R. E., Poff, A. M., and D'Agostino, D. P. (2014). Cancer as a metabolic disease: implications for novel therapeutics. *Carcinogenesis* 35 (3), 515–27. doi:10.1093/carcin/bgt480
- Shanab, A. A., Scully, P., Crosbie, O., Buckley, M., O'Mahony, L., Shanahan, F., et al. (2011). Small intestinal bacterial overgrowth in nonalcoholic steatohepatitis: association with toll-like receptor 4 expression and plasma levels of interleukin 8. *Dig. Dis. Sci.* 56, 1524–34. doi:10.1007/s10620-010-1447-3
- Shankar, E., Zhang, A., Franco, D., and Gupta, S. (2017). Betulinic acid-mediated apoptosis in human prostate cancer cells involves p53 and nuclear factor-kappa B (NF-κB) pathways. *Molecules* 22 (2), 264. doi:10.3390/molecules22020264
- Shaw, E., Farris, M., McNeil, J., and Friedenreich, C. (2016). Obesity and endometrial cancer. *Obes. Cancer* 208, 107–36. doi:10.1007/978-3-319-42542-9_7
- Shin, J., Lee, H.-J., Jung, D.-B., Jung, J. H., Lee, H.-J., Lee, E.-O., et al. (2011). Suppression of STAT3 and HIF-1 alpha mediates anti-angiogenic activity of betulinic acid in hypoxic PC-3 prostate cancer cells. *PLoS one* 6 (6), e21492. doi:10.1371/journal.pone.0021492
- Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., and Shulman, R. G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by ¹³C nuclear magnetic resonance spectroscopy. *N. Engl. J. Med.* 322 (4), 223–8. doi:10.1056/NEJM199001253220403
- Silva, F. S., Oliveira, P. J., and Duarte, M. F. (2016). Oleanolic, ursolic, and betulinic acids as food supplements or pharmaceutical agents for type 2 diabetes: promise or illusion? *J. Agric. Food Chem.* 64 (15), 2991–3008. doi:10.1021/acs.jafc.5b06021
- Silva, A. T., Cerqueira, M. Jo, Prudêncio, C., Fernandes, M. H., Costa-Rodrigues, Jo, Teixeira, Ct, et al. (2019). Antiproliferative organic salts derived from betulinic acid: disclosure of an ionic liquid selective against lung and liver cancer cells. *ACS Omega* 4 (3), 5682–9. doi:10.1021/acsomega.8b03691
- Singh, S., Sharma, A. N., Murad, M. H., Buttar, N. S., El-Serag, H. B., Katzka, D. A., et al. (2013). Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. *Clin. Gastroenterology Hepatology* 11 (11), 1399–1412. doi:10.1016/j.cgh.2013.05.009
- Singh, M., Thrimawithana, T., Shukla, R., and Adhikari, B. (2020). Managing obesity through natural polyphenols: a review. *Future Foods* 1, 100002. doi:10.1016/j.fufo.2020.100002
- Sowers, J. L., Johnson, K. M., Conrad, C., Patterson, J. T., and Sowers, L. C. (2014). The role of inflammation in brain cancer. *Inflamm. Cancer* 816, 75–105. doi:10.1007/978-3-0348-0837-8_4
- Su, D., Gao, Y.-q., Dai, W.-b., Hu, Y., Wu, Y.-f., and Mei, Q.-x. (2017). Helicteric acid, oleanic acid, and betulinic acid, three triterpenes from *Helicteres angustifolia* L., inhibit proliferation and induce apoptosis in HT-29 colorectal cancer cells via suppressing NF-κB and STAT3 signaling. *Evidence-Based Complementary Altern. Med.* 2017 (1), 5180707. doi:10.1155/2017/5180707
- Sun, K., and Scherer, P. E. (2010). "Adipose tissue dysfunction: a multistep process," in *Novel Insights Into Adipose Cell Functions*, 67–75.
- Sun, Y.-F., Song, C.-K., Viernstein, H., Unger, F., and Liang, Z.-S. (2013). Apoptosis of human breast cancer cells induced by microencapsulated betulinic acid from sour jujube fruits through the mitochondria transduction pathway. *Food Chem.* 138 (2-3), 1998–2007. doi:10.1016/j.foodchem.2012.10.079
- Sun, L., Cao, J., Chen, K., Cheng, L., Zhou, C., Yan, B., et al. (2018). Betulinic acid inhibits stemness and EMT of pancreatic cancer cells via activation of AMPK signaling. *Int. J. Oncol.* 54 (1), 98–110. doi:10.3892/ijo.2018.4604
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA a Cancer J. Clin.* 71 (3), 209–49. doi:10.3322/caac.21660
- Suresh, C., Zhao, H., Gumbs, A., Chetty, C. S., and Bose, H. S. (2012). New ionic derivatives of betulinic acid as highly potent anti-cancer agents. *Bioorg. and Med. Chem. Lett.* 22 (4), 1734–8. doi:10.1016/j.bmcl.2011.12.102
- Tak, Y. J., and Lee, S. Y. (2020). Anti-obesity drugs: long-term efficacy and safety: an updated review. *World J. Men's Health* 39 (2), 208–221. doi:10.5534/wjmh.200010
- Tan, Y., Yu, R., and Pezzuto, J. M. (2003). Betulinic acid-induced programmed cell death in human melanoma cells involves mitogen-activated protein kinase activation. *Clin. Cancer Res.* 9 (7), 2866–75. Available online at: <https://pubmed.ncbi.nlm.nih.gov/12855667/>
- Tang, X., Zhang, Q., Shi, S., Yen, Y., Li, X., Zhang, Y., et al. (2010). Bisphosphonates suppress insulin-like growth factor 1-induced angiogenesis *via* the HIF-1alpha/VEGF signaling pathways in human breast cancer cells. *Int. J. Cancer* 126 (1), 90–103. doi:10.1002/ijc.24710
- Teng, J.-A., Wu, S.-G., Chen, J.-X., Li, Q., Peng, F., Zhu, Z., et al. (2016). The activation of ERK1/2 and JNK MAPK signaling by insulin/IGF-1 is responsible for the development of colon cancer with type 2 diabetes mellitus. *PLoS One* 11 (2), e0149822. doi:10.1371/journal.pone.0149822
- Tirrò, E., Massimino, M., Romano, C., Martorana, F., Pennisi, M. S., Stella, S., et al. (2020). Prognostic and therapeutic roles of the insulin growth factor System in glioblastoma. *Front. Oncol.* 10, 612385. doi:10.3389/fonc.2020.612385
- Tiwari, R., Puthli, A., Balakrishnan, S., Sapra, B., and Mishra, K. (2014). Betulinic acid-induced cytotoxicity in human breast tumor cell lines MCF-7 and T47D and its modification by topopherol. *Cancer investig.* 32 (8), 402–8. doi:10.3109/07357907.2014.933234
- Tong, Y., Xu, S., Huang, L., and Chen, C. (2022). Obesity and insulin resistance: pathophysiology and treatment. *Drug Discov. Today* 27 (3), 822–30. doi:10.1016/j.drudis.2021.11.001
- Turkoz, F. P., Solak, M., Petekkaya, I., Keskin, O., Kertmen, N., Sarici, F., et al. (2013). Association between common risk factors and molecular subtypes in breast cancer patients. *breast* 22 (3), 344–50. doi:10.1016/j.breast.2012.08.005
- Valenti, L., Al-Serri, A., Daly, A. K., Galmozzi, E., Rametta, R., Dongiovanni, P., et al. (2010). Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 51 (4), 1209–17. doi:10.1002/hep.23622
- Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324 (5930), 1029–33. doi:10.1126/science.1160809

- Verma, S., and Hussain, M. E. (2017). Obesity and diabetes: an update. *Diabetes and Metabolic Syndrome Clin. Res. and Rev.* 11 (1), 73–9. doi:10.1016/j.dsx.2016.06.017
- Vidal, A. C., Howard, L. E., Moreira, D. M., Castro-Santamaria, R., Andriole, Jr G. L., and Freedland, S. J. (2014). Obesity increases the risk for high-grade prostate cancer: results from the REDUCE study. *Cancer Epidemiol. Biomarkers and Prev.* 23 (12), 2936–42. doi:10.1158/1055-9965.EPI-14-0795
- Viji, V., Helen, A., and Luxmi, V. R. (2011). Betulinic acid inhibits endotoxin-stimulated phosphorylation cascade and pro-inflammatory prostaglandin E2 production in human peripheral blood mononuclear cells. *Br. J. Pharmacol.* 162 (6), 1291–303. doi:10.1111/j.1476-5381.2010.01112.x
- Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. *Jama* 282 (22), 2131–5. doi:10.1001/jama.282.22.2131
- Wallace, A. M., McMahon, A. D., Packard, C. J., Kelly, A., Shepherd, J., Gaw, A., et al. (2001). Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation* 104 (25), 3052–6. doi:10.1161/hc5001.101061
- Wang, X., Lu, X., Zhu, R., Zhang, K., Li, S., Chen, Z., et al. (2017). Betulinic acid induces apoptosis in differentiated PC12 cells via ROS-mediated mitochondrial pathway. *Neurochem. Res.* 42, 1130–40. doi:10.1007/s11064-016-2147-y
- Wang, L., Yan, Y., Wu, L., and Peng, J. (2023). Natural products in non-alcoholic fatty liver disease (NAFLD): novel lead discovery for drug development. *Pharmacol. Res.* 196, 106925. doi:10.1016/j.phrs.2023.106925
- Wang, K., Shang, J., Tao, C., Huang, M., Wei, D., Yang, L., et al. (2024). Advancements in betulinic acid-loaded nanoformulations for enhanced anti-tumor therapy. *Int. J. Nanomedicine* 19, 14075–103. doi:10.2147/IJN.S493489
- Warburg, O. (1956). On the origin of cancer cells. *Science* 123 (3191), 309–14. doi:10.1126/science.123.3191.309
- Weber, D., Zhang, M., Zhuang, P., Zhang, Y., Wheat, J., Currie, G., et al. (2014). The efficacy of betulinic acid in triple-negative breast cancer. *SAGE Open Med.* 2. doi:10.1177/2050312114551974
- Wei, L., Li, K., Pang, X., Guo, B., Su, M., Huang, Y., et al. (2016). Leptin promotes epithelial-mesenchymal transition of breast cancer via the upregulation of pyruvate kinase M2. *J. Exp. and Clin. Cancer Res.* 35, 166–10. doi:10.1186/s13046-016-0446-4
- Weichhaus, M., Broom, J., Wahle, K., and Bermano, G. (2012). A novel role for insulin resistance in the connection between obesity and postmenopausal breast cancer. *Int. J. Oncol.* 41 (2), 745–52. doi:10.3892/ijo.2012.1480
- Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Investigation* 112 (12), 1796–808. doi:10.1172/JCI19246
- Weyer, C., Bogardus, C., Mott, D. M., and Pratley, R. E. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J. Clin. Investigation* 104 (6), 787–94. doi:10.1172/JCI7231
- WHO (2025). Obesity and overweight. Available online at: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.
- WHO. Obesity (2025). Available online at: https://www.who.int/health-topics/obesity#tab=tab_1.
- Wiechert, M., and Holzapfel, C. (2021). Nutrition concepts for the treatment of obesity in adults. *Nutrients* 14 (1), 169. doi:10.3390/nu14010169
- Wilcox, G. (2005). Insulin and insulin resistance. *Clin. Biochem. Rev.* 26 (2), 19–39. doi:10.1016/S0025-7125(03)00128-7
- Willis, S. A., Bawden, S. J., Malaikah, S., Sargeant, J. A., Stensel, D. J., Aithal, G. P., et al. (2021). The role of hepatic lipid composition in obesity-related metabolic disease. *Liver Int.* 41 (12), 2819–35. doi:10.1111/liv.15059
- Wolin, K. Y., Carson, K., and Colditz, G. A. (2010). Obesity and cancer. *Oncol.* 15 (6), 556–65. doi:10.1634/theoncologist.2009-0285
- Wu, H., and Ballantyne, C. M. (2020). Metabolic inflammation and insulin resistance in obesity. *Circulation Res.* 126 (11), 1549–64. doi:10.1161/CIRCRESAHA.119.315896
- Wu, S., Zhu, W., Thompson, P., and Hannun, Y. A. (2018). Evaluating intrinsic and non-intrinsic cancer risk factors. *Nat. Commun.* 9 (1), 3490. doi:10.1038/s41467-018-05467-z
- Xie, R., Zhang, H., Wang, X.-z., Yang, X.-z., Wu, S.-n., Wang, H.-g., et al. (2017). The protective effect of betulinic acid (BA) diabetic nephropathy on streptozotocin (STZ)-induced diabetic rats. *Food and Funct.* 8 (1), 299–306. doi:10.1039/c6fo01601d
- Xu, L., Zhou, R., Yuan, L., Wang, S., Li, X., Ma, H., et al. (2017). IGF1/IGF1R/STAT3 signaling-inducible IFITM2 promotes gastric cancer growth and metastasis. *Cancer Lett.* 393, 76–85. doi:10.1016/j.canlet.2017.02.014
- Yang, W. S., Chadalapaka, G., Cho, S.-G., Lee, S.-o., Jin, U.-H., Jutooru, I., et al. (2014). The transcriptional repressor ZBTB4 regulates EZH2 through a MicroRNA-ZBTB4-specificity protein signaling axis. *Neoplasia* 16 (12), 1059–69. doi:10.1016/j.neo.2014.09.011
- Yao, P., and Liu, Y. (2022). Terpenoids: natural compounds for non-alcoholic fatty liver disease (NAFLD) therapy. *Molecules* 28 (1), 272. doi:10.3390/molecules28010272
- Yaizu, Z., Liu, Y., Zhao, H., Peng, P., Tingbao, Z., and Jincao, C. (2020). Betulinic acid inhibits glioma cell viability by downregulation of NF- κ B and enhancement of apoptosis. *Trop. J. Pharm. Res.* 19 (12), 2545–51. doi:10.4314/tjpr.v19i12.9
- Ye, P., Xi, Y., Huang, Z., and Xu, P. (2020). Linking obesity with colorectal cancer: epidemiology and mechanistic insights. *Cancers* 12 (6), 1408. doi:10.3390/cancers12061408
- Yeh, W. L., Lu, D. Y., Lee, M. J., and Fu, W. M. (2009). Leptin induces migration and invasion of glioma cells through MMP-13 production. *Glia* 57 (4), 454–64. doi:10.1002/glia.20773
- Yoon, J. J., Lee, Y. J., Kim, J. S., Kang, D. G., and Lee, H. S. (2010). Protective role of betulinic acid on TNF- α -induced cell adhesion molecules in vascular endothelial cells. *Biochem. biophysical Res. Commun.* 391 (1), 96–101. doi:10.1016/j.bbrc.2009.11.009
- Yoon, J. J., Lee, Y. J., Han, B. H., Choi, E. S., Kho, M. C., Park, J. H., et al. (2017). Protective effect of betulinic acid on early atherosclerosis in diabetic apolipoprotein-E gene knockout mice. *Eur. J. Pharmacol.* 796, 224–32. doi:10.1016/j.ejphar.2016.11.044
- Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64 (1), 73–84. doi:10.1002/hep.28431
- Yun, Y., Han, S., Park, E., Yim, D., Lee, S., Lee, C.-K., et al. (2003). Immunomodulatory activity of betulinic acid by producing pro-inflammatory cytokines and activation of macrophages. *Archives pharmacol. Res.* 26, 1087–95. doi:10.1007/BF02994763
- Zeng, A.-Q., Yu, Y., Yao, Y.-Q., Yang, F.-F., Liao, M., Song, L.-J., et al. (2017). Betulinic acid impairs metastasis and reduces immunosuppressive cells in breast cancer models. *Oncotarget* 9 (3), 3794–3804. doi:10.18632/oncotarget.23376
- Zeng, A., Hua, H., Liu, L., and Zhao, J. (2019). Betulinic acid induces apoptosis and inhibits metastasis of human colorectal cancer cells *in vitro* and *in vivo*. *Bioorg. and Med. Chem.* 27 (12), 2546–52. doi:10.1016/j.bmc.2019.03.033
- Zhan, X. K., Li, J. L., Zhang, S., Xing, P. Y., and Xia, M. F. (2018). Betulinic acid exerts potent antitumor effects on paclitaxel-resistant human lung carcinoma cells (H460) via G2/M phase cell cycle arrest and induction of mitochondrial apoptosis. *Oncol. Lett.* 16 (3), 3628–34. doi:10.3892/ol.2018.9097
- Zhang, X., Zhou, G., Sun, B., Zhao, G., Liu, D., Sun, J., et al. (2015). Impact of obesity upon prostate cancer-associated mortality: a meta-analysis of 17 cohort studies. *Oncol. Lett.* 9 (3), 1307–12. doi:10.3892/ol.2014.2841
- Zhang, N., Liu, X., Zhuang, L., Liu, X., Zhao, H., Shan, Y., et al. (2020). Berberine decreases insulin resistance in a PCOS rats by improving GLUT4: dual regulation of the PI3K/AKT and MAPK pathways. *Regul. Toxicol. Pharmacol.* 110, 104544. doi:10.1016/j.yrtph.2019.104544
- Zhao, Z., Kosinska, W., Khmelnskiy, M., Cavalieri, E. L., Rogan, E. G., Chakravarti, D., et al. (2006). Mutagenic activity of 4-hydroxyestradiol, but not 2-hydroxyestradiol, in BB rat2 embryonic cells, and the mutational spectrum of 4-hydroxyestradiol. *Chem. Res. Toxicol.* 19 (3), 475–9. doi:10.1021/tx0502645
- Zhao, Y., He, X., Shi, X., Huang, C., Liu, J., Zhou, S., et al. (2010). Association between serum amyloid A and obesity: a meta-analysis and systematic review. *Inflamm. Res.* 59, 323–34. doi:10.1007/s00011-010-0163-y
- Zhao, S., Kusminski, C. M., and Scherer, P. E. (2021). Adiponectin, leptin and cardiovascular disorders. *Circulation Res.* 128 (1), 136–49. doi:10.1161/CIRCRESAHA.120.314458
- Zhao, H., Wu, L., Zhang, Y., Feng, S., Ding, Y., Deng, X., et al. (2022). Betulinic acid prevents liver fibrosis by binding Lck and suppressing Lck in HSC activation and proliferation. *J. Ethnopharmacol.* 296, 115459. doi:10.1016/j.jep.2022.115459
- Zhu, X., Wang, D., and Atanasov, A. G. (2024). “A review: Anti-obesity drug discovery from natural plant metabolites and endogenous peptides,” in *Current Research In Biotechnology*. 100259.
- Zuco, V., Supino, R., Righetti, S. C., Cleris, L., Marchesi, E., Gambacorti-Passerini, C., et al. (2002). Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. *Cancer Lett.* 175 (1), 17–25. doi:10.1016/s0304-3835(01)00718-2