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The hepatoprotective effects of silkworm: Insights into molecular mechanisms and implications

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Abstract

The liver, a multifunctional organ, plays a vital role in maintaining overall health and well-being by regulating metabolism, detoxification, nutrient storage, hormone balance, and immune function. Liver diseases, such as hepatitis, cirrhosis, fatty liver disease, and liver cancer, have significant clinical implications and remain a global health concern. This article reviews the therapeutic potential of silkworm larvae (*Bombyx mori*) and explores their underlying molecular mechanisms in protecting against liver diseases. Silkworm larvae are rich in proteins, vitamins, minerals, and n-3 fatty acids, making them a promising candidate for therapeutic applications. The anti-inflammatory mechanisms of silkworm larvae involve modulating the production of cytokine such as TNF-α and interleukins, inflammatory enzymes including cyclooxygenase-2 and macrophage polarization, thereby attenuating liver inflammation. Silkworm larvae also exhibit anti-oxidative effects by scavenging free radicals, reducing intracellular reactive oxygen species and enhancing the liver's antioxidant defense system. Moreover, silkworms have been reported to decrease the serum alcohol concentration and lipid accumulation. Understanding the therapeutic properties of silkworm larvae contributes to the development of innovative strategies for liver injury prevention and treatment. Further research is warranted to elucidate the precise signaling pathways involved in the anti-inflammatory and anti-oxidative effects of silkworm larvae, paving the way for potential therapeutic interventions in liver diseases.

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Introduction

The liver, an organ of exceptional significance, plays a central role in maintaining overall health and well-being. Situated in the upper right side of the abdomen, this complex organ performs an astonishing array of vital functions, making it an indispensable component of the human body. Its multifaceted roles encompass metabolism, detoxification, nutrient storage, hormone regulation, and immune function, among others (Acharya *et al.*, 2021). The liver's primary function revolves around metabolic processes that impact the entire body (Rui, 2014). Through intricate biochemical pathways, the liver facilitates the production of essential molecules, including glucose, glycogen, cholesterol, and bile acids (Schulze *et al.*, 2019). Additionally, it plays a pivotal role in detoxification by filtering and eliminating potentially harmful substances from the bloodstream, including drugs, toxins, and metabolic waste products (Schulze *et al.,* 2019).

Beyond its metabolic functions, the liver serves as a critical

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site for the storage of various essential nutrients and vitamins. It reserves glycogen as a readily available energy source, ensuring a steady supply of glucose during fasting periods (Koretz, 2023). The liver also stores essential fat-soluble vitamins such as vitamins A, D, E, and K, which are released into circulation as needed, contributing to the overall nutritional balance of the body (Elsebaie *et al.*, 2023). Furthermore, the liver acts as a regulator of hormonal balance through the metabolism and clearance of hormones, including insulin, glucagon, and various sex hormones (Rhyu and Yu, 2021). It maintains optimal hormonal levels, ensuring their efficient signaling and exertion of physiological effects throughout the body. Given the liver's indispensable contributions to overall health, any disruption or dysfunction of this organ can lead to severe medical conditions and compromise bodily functions (Elsebaie *et al.*, 2023). Liver diseases, encompassing a wide spectrum of disorders such as hepatitis, cirrhosis, fatty liver disease, and liver cancer, have significant clinical implications and remain a major global health concern (Moon *et al.*, 2020). Understanding the mechanisms underlying liver diseases, as well as developing effective diagnostic tools and therapeutic strategies, are paramount in combating these conditions and improving patient outcomes.

The silkworm, *Bombyx mori*, has been renowned for its role in silk production since ancient times (Lee *et al.*, 2017a). However, recent studies have highlighted its potential as a source of beneficial compounds with significant health effects (Ji *et al.*, 2016b). The larvae of *Bombyx mori*, in particular, are rich in crude proteins, vitamins, minerals, and n-3 fatty acids, making them a promising candidate for therapeutic applications in various conditions, including Parkinson's disease, hyperlipidemia, hyperglycemia, liver diseases, and gastrointestinal disorders (Kim *et al.*, 2008; Tabunoki *et al.*, 2013; Ji *et al.,* 2016b). The aim of this review is to shed light on the therapeutic potential of silkworm larvae and elucidate the underlying molecular mechanisms responsible for their protective effects in liver diseases. By providing a comprehensive understanding of the beneficial properties of *Bombyx mori* larvae, we aim to contribute to the development of innovative therapeutic and preventive strategies for liver injury and hepatic disorders.

Anti-Inflammatory mechanisms of silkworms

Liver inflammation has been recognized for its hepatoprotective effects, as it safeguards hepatocytes from damage, facilitates tissue repair, and aids in the restoration of homeostasis (Waidmann *et al.*, 2013). However, excessive liver inflammation can lead to liver cell damage, resulting in irreversible liver damage, hepatitis, fibrosis, cirrhosis, and liver cancer (Waidmann *et al.,* 2013).

In non-alcoholic fatty liver disease (NAFLD) and alcoholic liver injury, inflammation is primarily driven by macrophages (Mosser and Edwards, 2008; Zhang *et al.*, 2016). Given the liver's central role in lipid and glucose metabolism, hepatic inflammation contributes to hepatic metabolic disorders (Browning and Horton, 2004). Furthermore, chronic liver inflammation-induced hepatocellular damage triggers hepatic fibrosis mediated by the activation of hepatic stellate cells (HSCs) and excessive extracellular matrix (ECM) secretion (Gluchowski *et al.*, 2017). Activated HSCs exacerbate liver inflammation by promoting ECM production and cytokine secretion in non-alcoholic steatohepatitis (NASH)-induced liver fibrosis (Gluchowski *et al.,* 2017). Persistent inflammation can lead to the release of inflammatory mediators such as IL-6 and TNF-α, which are reported to exacerbate liver damage and contribute to the formation of malignant tumors (Lin and Karin, 2007; Fukata and Abreu, 2008). Leptin, an adipocyte-derived hormone associated with obesity, plays a role in mediating liver inflammation through one of the signaling pathways (Potter *et al.*, 2003). Indeed, leptin receptor-deficient mice were protected from carbon tetrachloride (CCl4)-induced liver fibrosis, and leptin-stimulated Kupffer cells were found to activate HSCs (Ikejima *et al.*, 2002). Additionally, hepatic inflammation is mediated through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway (Karin, 2006). Knockdown of the inhibitor of nuclear factor kappa-B kinase subunit (IKK)-β in Kupffer cells significantly suppressed tumorigenesis in a chemically induced liver cancer model (Maeda *et al.*, 2005). Furthermore, the removal of the IKK complex IKKγ/NEMO in hepatocytes inhibited NF-kB activity, impeding the progression of chronic hepatitis and liver cancer (Bettermann *et al.*, 2010).

Signal transducer and activator of transcription 3 (STAT3) activity is another mediator of hepatic inflammation, which can be induced by cytokines such as IL-6 through Janus kinase (JAK). The JAK-STAT pathway is activated in liver inflammation and liver cancer (Yu *et al.*, 2007). Moreover, various growth factor signaling pathways, including insulin-like growth factor 1 (IGF), hepatocyte growth factor (HGF), Wingless-related integration site (Wnt), TGF-β, and EGF, have been found to be involved in hepatitis (Breuhahn *et al.*, 2006). Several studies have

confirmed the hepatoprotective effects of silkworm powder (3rd day of the 5th instar larvae of Baekokjam) in liver inflammation (Hong *et al.*, 2018; Lee *et al.*, 2020a). Firstly, silkworm powder consumption for 4 weeks suppressed the serum concentration and mRNA levels of TNF-α in an alcohol-induced fatty liver rat model, indicating its inhibitory effect on liver inflammation (Hong *et al.,* 2018). Additionally, administration of silkworm powder reduced the concentrations of TNF-α and IL-1β in serum and alleviated the mRNA levels of IL-1β in an alcohol-induced fatty liver rat model (Lee *et al.,* 2020a). Interestingly, silkworm powder has also demonstrated liver damage and liver cancer prevention effects. In a diethylnitrosamine (DEN)-induced acute liver injury mouse model, silkworm powder inhibited macrophage and cluster of differentiation 31 (CD31) infiltration and reduced the expression of IL-1β, IFN-γ, and chemokines such as C-X-C motif chemokine ligand 11, C-C motif chemokine ligand 17, and C-C chemokine receptor type 2. It also suppressed the expression of inflammatory enzymes such as cyclooxygenase-2 (COX-2) (Cho *et al.*, 2016a). These effects of silkworm powder have been reported to inhibit liver damage, inflammatory cytokines, and reduce inflammatory cell infiltration (Cho *et al.*, 2016b). Furthermore, in a DEN-induced chronic liver cancer rat model, silkworm powder effectively reduced the levels of TNF-α and IL-1β in the serum and suppressed mRNA expression for 16 weeks. This effect of silkworm powder was reported to occur through the inhibition of STAT3 phosphorylation, an inflammatory signaling pathway (Lee *et al.,* 2020a). These beneficial effects of silkworm powder have been observed not only in the liver but also in the stomach. In an alcohol-induced acute gastric injury rat model, silkworm powder inhibited inflammation-related factors such as CD31 and F4/80 and reduced the expression of TNF-α, IL-1β, IL-6, IL-8, and IL-10 (Yun *et al.*, 2017). Even at a low concentration (100 mg/kg), silkworm powder inhibited inflammatory factors such as COX-2, TNF-α, and IL-1β in the same model (Lee *et al.*, 2023b). However, further research is required to elucidate the precise signaling pathway responsible for well-known anti-inflammatory effect of silkworm powder.

Anti-oxidative mechanisms of silkworms

During the development of larvae, which involves five stages (1st-5th instar), cellular processes (including cellular proliferation, differentiation and migration for pattern formation) occur that result in increased levels of oxidizing substances such as ROS and ROS-promoting plant allelochemicals (Sahoo *et al.*, 2016). The study indicates that 1st instar larvae, trivoltine strains of tasar silk worm (*A. mylitta*), are more susceptible to oxidative stress compared to older larvae, as evidenced by higher levels of malondialdehyde (MDA), the lipid peroxidation marker, and H_2O_2 (Sahoo *et al.,* 2016). This vulnerability may be attributed to factors such as exposure to ambient oxygen, ingestion of plant allelochemicals, and the requirement for H_2O_2-generating enzymes for cuticle formation (Sahoo *et al.,* 2016). As the larvae mature, their antioxidant defenses improve, with 2nd and 3rd instar larvae exhibiting a more efficient antioxidant profile (Sahoo *et al.,* 2016). Enzymatic antioxidants like SOD and catalase play a critical role in protecting 1st instar larvae, while both enzymatic and nonenzymatic antioxidants contribute to the defense system of older larvae (Sahoo *et al.,* 2016).

Furthermore, the digestion of silkworm proteins led to the release of hydrolysates containing bioactive peptides with enhanced antioxidant properties (Zhang *et al.*, 2021b). Another study investigated the radical scavenging effects of muga silkworm protein extract (MPE, from the Udalguri district of Assam, India) using the DPPH assay, showing a dose-dependent increase in scavenging activity as the concentration of MPE increased (Deori *et al.*, 2014). Additionally, silkworm pupae extract was found to reduce intracellular ROS levels in oxidatively stressed fibroblast cells (Rahul *et al.*, 2022). The results indicated a significant decrease in ROS production in cells pretreated with silkworm pupae extract compared to untreated cells (Rahul *et al.,* 2022). Moreover, silkworm pupa oil has been reported to possess antioxidant effects in an animal model of liver injury induced by acetaminophen (Long *et al.*, 2020). Acetaminophen treatment resulted in increased serum MDA levels, indicating the presence of ROS and lipid peroxidation in Kunming male mice model (Long *et al.,* 2020). However, supplementation of silkworm pupa oil dose-dependently decreased MDA levels and restored the activities of antioxidative enzymes such as SOD and plasma glutathione peroxidase (GSH-Px) (Long *et al.*, 2020). This enhanced antioxidant ability of silkworm pupa oil contributed to the reduction of hepatic inflammation and injury in acetaminophen-treated mice (Long *et al.*, 2020).

Previous research has focused on exploring the antioxidant properties of silkworm sericin, as well as silkworm extract and hydrolysates. Sericin, a macromolecular protein derived from the silkworm *Bombyx mori*, plays a crucial role in cocoon formation due to its sticky nature and high hydrophilic amino acid content (Zhang, 2002). The antioxidant activities of silk sericin have been

investigated, including its ability to scavenge hydroxyl radicals, superoxide radicals, and DPPH radicals, as well as inhibit lipid peroxidation (Fan *et al.*, 2009). The antioxidant effect of sericin likely plays a predominant role in the antioxidant capacity of the non-peptidoid component associated with the sericin fraction (Suzuki *et al.*, 2022). Investigators performed by separating into three fractions: two peptidoid fractions, the cude sericin and the purified sericin, and the non-peptidoid methanolic extract of the crude fraction (Suzuki *et al.*, 2022). Assessment of antioxidant activity using a trolox equivalent anti-oxygen capacity (TEAC) assay and cultured murine retinal photoreceptor cells revealed the highest level of antioxidant activity at the cellular level, particularly with the non-peptidoid components associated with sericin, which were extracted from the crude sericin using a solvent (Suzuki *et al.,* 2022). Although the crude sericin also exhibited some antioxidant activity at higher concentrations, the sericin-associated non-peptidoid components displayed the most significant antioxidative capacity (Suzuki *et al.,* 2022).

In our previous study, it was found that silkworm powder (Baekokjam) exhibits antioxidant effects in an ethanol-induced gastric injury Sprague-Dawley rat model (Yun *et al.*, 2017). Ethanol treatment (for 3 hours) led to increased oxidative stress, as indicated by decreased levels of total oxidant concentration (Yun *et al.,* 2017). However, pretreatment with silkworm powder (1g/kg/body weight, for 2 weeks) significantly restored the antioxidant capacity and reduced the levels of MDA (Yun *et al.,* 2017). These findings suggest that silkworm powder pretreatment can alleviate ethanol-induced damage by attenuating oxidative stress and improving antioxidant defenses (Yun *et al.,* 2017). Although direct research on the antioxidant function of silkworm in liver disease is insufficient, it is hypothesized that silkworm possesses significant antioxidant properties that may contribute to liver protection.

The effects of silkworms on alcohol metabolism

Some of the health functional foods produced these days use silkworms (Ji *et al.,* 2016b). Additionally, there is a report that the silk fibers of silkworms significantly reduce alcohol absorption levels (Kunz and Brancalhao, 2016). When silkworm larvae mature, silkworms may contain different nutrients, and silk threads of different colors are produced by the accumulation of certain plant chemicals in the silkworm glands (Kang *et al.*, 2007). Therefore, the nutrients contained in mature silkworm powder vary according to the breed of silkworm (Ji *et al.*, 2016a). Silkworm powder contains a large amount of amino acids, essential minerals and crude proteins, and with the development of techniques for manufacturing mature silkworm larvae to steamed and freeze-dried mature silkworm larval powder it is easier to eat than before (Ji *et al.,* 2016a; Ji *et al.,* 2016b). Mice fed with 1 g/kg of Baekokjam, 1 g/kg of Golden-silk and 1 g/kg of Yeonnokjam silkworm powder for two weeks, which were obtained from white, golden, and light green cocoons respectively, showed reduced ethanol-induced ADH and ALDH activities than mice fed a normal diet (Lee *et al.*, 2017b). In particular, Baekokjam silkworm powder showed greater inhibitory efficacy than other silkworm powders (Lee *et al.,* 2017b). In addition, among the three silkworm powders, Baekokjam silkworm powder was the most effective in inhibiting the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which are indicators of liver damage, and their activity increases when alcohol consumption increases in ethanol-treated rats (Lee *et al.,* 2017b). Treatment with silkworm powder significantly reduced blood alcohol and acetaldehyde levels and demonstrated protective effects against liver damage (Lee *et al.,* 2017b). Baekokjam silkworm powder is described as having a protective effect against ethanol-induced liver damage due to its higher content of beneficial amino acids such as glycine, alanine, and tyrosine compared to than Golden-silk and Yeonnokjam silkworm powder (Lee *et al.,* 2017b). Therefore, Baekokjam silkworm powder can be used as a promising candidate for improving alcohol metabolism and relieving hangovers (Lee *et al.,* 2017b).

Effects of silkworms on signaling in liver diseases

Fatty liver diseases

The pathogenesis of fatty liver is crucially involved in the regulation of fatty acid synthesis, oxidation, and extraction mechanisms. Notably, pivotal regulatory factors including sterol regulatory element binding transcription (SREBP) 1, peroxisome proliferator-activated receptor (PPAR)-α/γ, sirtuin (SIRT)-1, and AMP-activated protein kinase (AMPK) have been implicated in the modulation of lipogenesis and fatty acid oxidation (Reddy and Sambasiva Rao, 2006; Rasineni and Casey, 2012; Zhang *et al.*, 2021a). AMPK is an important regulator in energy metabolism and is involved in the modulation of fatty acid oxidation and synthesis (Long and Zierath, 2006). In fatty acid synthesis, AMPK inhibits the enzyme acetyl-CoA carboxylase (ACC) by phosphorylating it, thereby inactivating it. ACC is responsible for converting acetyl-CoA to malonyl-CoA, which

is an important precursor in fatty acid synthesis. By inhibiting ACC, AMPK reduces the availability of malonyl-CoA, leading to a decrease in fatty acid synthesis (Witters and Kemp, 1992). On the other hand, AMPK promotes fatty acid oxidation by the inhibitory phosphorylation of ACC1, a subunit of ACC, thereby AMPK enhances the breakdown of fatty acids to produce energy. Adiponectin stimulates hepatic AMPK which in turn phosphorylates ACC on Ser-79 and attenuates ACC activity. Inhibition of ACC directly reduces lipid synthesis and indirectly enhances fatty acid oxidation (Rogers *et al.*, 2008). Recent studies have reported that SIRT1, an NAD+-dependent protein deacetylase, plays crucial roles in regulating lipid metabolism and fatty liver diseases (Lieber *et al.*, 2008; Lee *et al.*, 2020a). Activation of SIRT1 inhibits lipogenesis by deacetylating ChREBP and SREBP-1c (Cantó and Auwerx, 2009), promotes fatty acid β-oxidation by deacetylating PPARα/PGC-1α (Purushotham *et al.*, 2009), defends against hepatic oxidative stress by enhancing antioxidant capability by deacetylating FOXOs and PGC-1α (Lagouge *et al.*, 2006). Additionally, SIRT1 reduces inflammation in the liver by deacetylating NF-κB, a transcription factor involved in inflammatory signaling (Tian *et al.*, 2016).

Interestingly, several studies have indicated the hepatoprotective effects of silkworm powder in fatty liver diseases (Hong *et al.*, 2018; Lee *et al.,* 2020a; Lee *et al.*, 2023a). Alcohol consumption causes liver damage including fatty liver, fibrosis, cirrhosis and hepatocellular carcinoma. In particular, alcoholic liver disease causes the accumulation of ROS, further promoting the secretion of pro-inflammatory cytokines (O'Shea *et al.*, 2010; Louvet and Mathurin, 2015). In an ethanol-induced liver damage rat model, silkworm powder (Baekokjam) suppressed hepatic steatosis and lipogenesis by regulating lipid metabolism (Hong *et al.,* 2018; Lee *et al.,* 2020a). Also, silkworm powder enhanced fatty acid oxidation and inhibited lipid synthesis, thereby preventing the accumulation of hepatic lipid through the SIRT-AMPK-ACC signaling pathway in ethanol-treated rats (Hong *et al.*, 2018; Lee *et al.,* 2020a). Administration of silkworm powder (Baekokjam) increased the mRNA expression of SIRT1 and significantly reduced the protein and mRNA levels of SREBP1 (Hong *et al.,* 2018; Lee *et al.,* 2020a). Moreover, silkworm powder significantly restored the phosphorylation of AMPK-α1/2 and the phosphorylation of ACC, a downstream substrate of the AMPK signaling pathway (Hong *et al.*, 2018; Lee *et al.*, 2020a). Furthermore, the hepatoprotective effect of silkworm powder (3rd and 5th instar) against non-ethanol-induced liver diseases, silkworm powder inhibited lipid accumulation and reduced the expression of PPAR-γ, SREBP-1, CCAAT/enhancer binding proteins-α, and fatty acid synthase by increasing the phosphorylation of AMPK and ACC based on *in vitro* and *in vivo* experiments (Park *et al.*, 2021).

Hepatic fibrosis

Hepatic fibrosis is characterized by the proliferation of HSCs, which are primarily responsible for the aberrant accumulation of extracellular matrix in the liver. This pathological process is accompanied by the activation of various signaling pathways that drive the progression of liver fibrosis (Tsukada *et al.*, 2006). Several studies have emphasized the pivotal role of signaling cascades, such as TGF-β and NF-κB, in regulating the fibrotic response of HSCs (Friedman, 1999; Lang *et al.*, 2000; Gressner *et al.*, 2002). TGF-β is a multifunctional growth factor that exerts a crucial role in activating fibrosis and stimulating the synthesis and deposition of components (Shek and Benyon, 2004). The TGF-β signaling cascade predominantly involves the activation of mothers against decapentaplegic homolog (Smad) 2 and Smad3 proteins. Upon binding to its TGF-β type II receptor, TGF-β initiates the phosphorylation of the TGF-β type I receptor, subsequently leading to the phosphorylation of Smad2/3 proteins (Yun *et al.*, 2022). The phosphorylated Smad2/3 complex is then translocated to the nucleus, where it governs the transcription of EMT-related target genes such as E-cadherin, N-cadherin, vimentin, α-smooth muscle actin (Meng and Nikolic-Paterson, 2016).

Importantly, several studies have elucidated the mechanisms underlying the anti-fibrotic effects of silkworm powder in fatty liver diseases. Silkworm powder (Baekokjam) exhibited significant reductions in the production and deposition of collagen fiber in both ethanol-induced liver damage rat model and DEN-induced chronic liver cancer rat model *in vivo*. Additionally, silkworm powder (Baekokjam) sattenuated the protein and mRNA levels of pro-fibrotic markers such as type 1 collagen and α-smooth muscle actin (Hong *et al.,* 2018; Lee *et al.*, 2020b). Notably, anti-fibrotic effect of silkworm powder (Baekokjam) was investigated through the TGF-β/SMAD signaling pathway in a DEN-induced rat model (Lee *et al.,* 2020b). Silkworm powder (Baekokjam) effectively inhibited the protein levels of TGF-β receptor I and the phosphorylation of Smad3. Furthermore, silkworm powder (Baekokjam) suppressed the mRNA expression levels of TGF-β, plasminogen activator

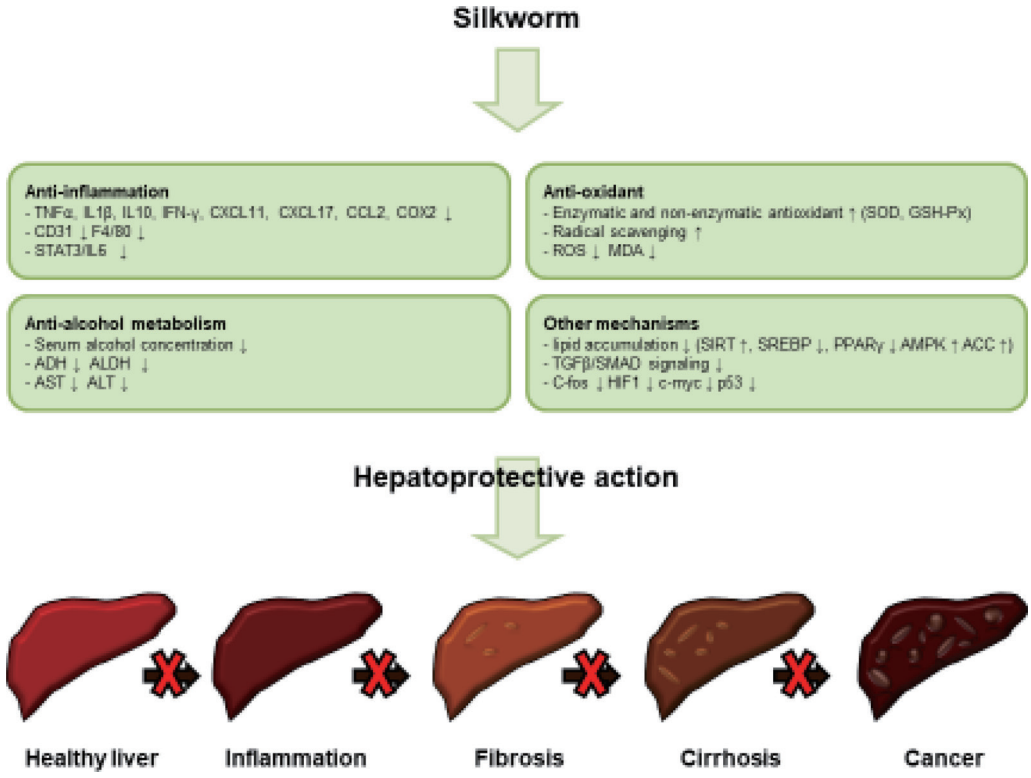

Fig. 1. Schematic representation summarizing the hepatoprotective action of silkworms through different mechanisms.

inhibitor-1, and connective tissue growth factor by inhibiting TGF-β/SMAD signaling pathway (Lee *et al.,* 2020b).

Liver cancer

The activation of STAT3 is triggered by cytokines or growth factors, including IL-6. Under normal circumstances, STAT3 remains in the cytoplasm (Svinka *et al.*, 2014; Xu *et al.*, 2021). However, upon activation, STAT3 is phosphorylated, forms dimers with other STAT family members, and translocates to the nucleus. In the nucleus, it initiates the transcription of target genes, including cyclin D1, B-cell lymphoma-extra large, c-myc, induced myeloid leukemia cell differentiation protein 1, and vascular endothelial growth factor (VEGF) (Svinka *et al.*, 2014; Xu *et al.,* 2021). In terms of liver cancer, STAT3 activation transcriptionally induces EMT markers, including Slug and Twist, and induces invasion and metastasis through the mediation of EMT in hepatocellular carcinoma (HCC) (Kang *et al.*, 2015). Mechanistically, STAT3 upregulates and recruits hypoxia-inducible factor (HIF)-1α to form a transcriptional complex that binds to the VEGF promoter, thereby inducing VEGF expression (Xu *et al.*, 2005). Additionally, STAT3 can promote stemness in HCC cells by activating Notch signaling, which is implicated in the self-renewal and proliferation of cancer stem cells (CSCs) (Xiong *et al.*, 2018). Furthermore, the studies revealed that cooperation between STAT3 and NF-kB, with approximately one-third of HCC tumors displaying concomitant activation of STAT3 and NF-kB (Crusz and Balkwill, 2015). Notably, silkworm powder (Baekokjam) exhibited significant reductions in carcinogenesis through the STAT3 signaling pathway in DEN-induced chronic liver cancer rat model (Cho *et al.*, 2019; Lee *et al.,* 2020b). It decreased STAT3 phosphorylation, suppressed IL-6 mRNA levels in liver cancer, and attenuated the expression of hepatic carcinogen-related target genes, such as c-fos, HIF-1, c-Myc, p53, and organic cation transporter-1 (Lee *et al.,* 2020b). Furthermore, *in vitro* experiments using human hepatocellular carcinoma cells demonstrated the anti-cancer effects of silkworm larva through the mitochondrial apoptosis pathway. Silkworm larva increased the protein levels of B-cell leukemia/lymphoma-2 (BCL-2), BCL2 associated X (BAX), caspases-8, -9, and -3, while suppressing the levels of Bcl-2 proteins in these cells (Cho *et al.,* 2019).

Conclusion

In conclusion, the manuscript provides a comprehensive

overview of the therapeutic potential of silkworm larvae and their underlying molecular mechanisms for protecting against liver diseases. The liver, being a crucial organ involved in various metabolic processes, is susceptible to inflammation and oxidative stress, which can lead to severe medical conditions. The review highlights the anti-inflammatory mechanisms of silkworm larvae, emphasizing their ability to suppress pro-inflammatory signals and regulate the balance between M1 and M2 macrophages. Silkworm larvae also demonstrate anti-oxidative properties through their rich content of antioxidants, including proteins and peptides, which scavenge free radicals and protect against oxidative damage. The findings suggest that silkworm larvae could be a promising candidate for therapeutic interventions in liver diseases, including fatty liver, hepatitis, and liver cancer. Further research is needed to elucidate the precise signaling pathways responsible for the anti-inflammatory and anti-oxidative effects of silkworm larvae. Overall, this manuscript contributes to the development of innovative preventive and therapeutic strategies for liver injury and hepatic disorders, offering potential avenues for improving patient outcomes in the field of liver disease research.

Conflict of Interests

All authors have nothing to disclose and have no commercial or financial interest in the products described in this paper.

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