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Green Alternatives in Treatment of Liver Diseases: the Challenges of Traditional Medicine and Green Nanomedicine

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Over the last decade, liver diseases have become a global problem, with approximately two million deaths per year. The high increase in the mortality rate of these diseases is mostly related to the limitations in the understanding of the evolutionary clinical cases of liver diseases, the low delivery of drugs in the liver, the non-specific administration of drugs, and the side effects generated at the systemic level by conventional therapeutic agents. Today it is common knowledge that phytochemicals have a high curative potential, even in the prevention and/or reversibility of liver disorders; however, even using these green molecules, researchers continue to deal with the same challenges implemented with conventional therapeutic agents, which limits the pharmacological potential of these friendly molecules. On the other hand, the latest advances in nanotechnology have proven that the use of nanocarriers as a delivery system for green active ingredients, as well as conventional ones, increases the pharmacological potential of these active ingredients due to their physicochemical characteristics (size, Zeta potential, etc.,) moldable depending on the therapeutic objective; in addition to the above, it should be noted that in recent years, nanoparticles have been developed for the specific delivery of drugs towards a specific target (stellar cells, hepatocytes, Kupffer cells), depending on the clinical state of the disease in the patient. The present review addresses the challenges of traditional medicine and green nanomedicine as alternatives in the treatment of liver diseases.

1. Introduction

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Important: Please take attention to italic and boldface as well as to the quotation marks in the article title.■■ The liver is the center of numerous physiological processes, including nutrient metabolism, immune system support, lipid and cholesterol homeostasis, and the breakdown of xenobiotic compounds;[1] due to its multiple functions, the liver is in contact with exogenous and systemic substances continuously and is therefore highly exposed to injury and possible development of acute and/or chronic diseases.[2,3] The main damaging factors of the liver are viruses, high alcohol and drug consumption, an unbalanced diet, and metabolic and autoimmune diseases. Indeed, liver diseases constitute a major health problem, as they endanger human life;[4] these etiologies, together or individually, can trigger a cascade of liver diseases, from simple cellular damage to cirrhosis and even liver cancer.

Due to the complex hepatic pathophysiology and the different progression levels of these conditions, developing a drug for their treatment remains a major challenge for modern pharmacology.[5] For the first clinical cases of liver diseases alcoholic liver disease (ALD) and Non-Alcoholic Fatty Liver Disease (NAFLD), the therapeutic approaches to reverse the

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progression of the disease are based on lifestyle changes and the use of insulin-regulating drugs to reduce the number of adipocytes, i.e., to reduce body weight.[6] For more complex clinical cases such as fibrosis and cirrhosis, treatment may vary depending on the stage of the disease and the underlying etiology. For fibrosis, treatment consists of reversing and preventing decompensation, while the treatment of cirrhosis consists of preventing decompensation, death and treating portal hypertension (to avoid progression to a more serious clinical condition, such as hepatic carcinoma).[7] Specifically, Entecavir is one of the recurrent drugs for the reversal of fibrosis/cirrhosis in patients with chronic hepatitis B, which can be applied for 3 to 7 years in patients to achieve a successful outcome.[8] In cases of acute cirrhosis/decompensated cirrhosis, it is reported that its treatment consists of limiting the progression of the disease, restricting/avoiding the involvement of other organs (kidney, heart, etc.); therefore, treatment includes i) treatment of microbiome abnormalities and bacterial translocation (e.g., Rifaximin); ii) improving abnormal circulatory function (e.g., long-term albumin); iii) treating inflammation (e.g., GS-0976, Statins, etc.); iv) addressing portal hypertension (e.g., antivirals), v) limiting oxidative stress (e.g., Losartan); vi) inhibition of hepatic apoptosis (e.g., Selonsertib, β-elemene).[7,8]

On the other hand, numerous studies have proven the benefits that specific phytochemicals can provide as an alternative to prevent and/or reverse liver diseases, with reduced adverse effects.[9] However, due to factors such as low solubility and stability, low absorption, and bioavailability *in situ*, the medicinal potential of these natural compounds is highly affected, which consequently limits their therapeutic efficacy.[10] Furthermore, conventional dosage forms (emulsions, tablets, capsules, etc.) do not allow to take full advantage of the pharmacological potential provided by the phytoconstituents of medicinal plants. In addition, advances in nanomedicine have led to the development of improved ways of dosing biologically active green molecules by using nanocarriers as a transport vehicle to enhance the absorption and bioavailability of these active compounds in the hepatic environment to carry out their therapeutic function. Nowadays, it is known that the use of nanoparticles in the field of pharmacology allows the controlled release of active molecules, increases the bioavailability or bio-accumulation of the drug in the target site, increases the circulation time of the active, and even allows overcoming several biological barriers of the body, which commonly prevent the active molecules from reaching the target and fulfilling their pharmacological function.[11] This cluster of advantages of nanoparticles enhances the biological effect of active drug molecules and limits the potential adverse effects commonly seen in conventional dosage forms of drug delivery.

Accordingly, this review aims to highlight the role of medicinal plants as treatment and/or green adjuvant in the therapy of liver diseases of different etiology; it also discusses and hypothesizes solutions from the nanotechnological point of view, through the use of green nanomedicines (nanoparticles loaded with plant extracts or phytocompounds) specifically designed to increase the pharmacokinetics, bio-accumulation, controlled release and oriented to a specific target (target liver cell) of green active ingredient.

2. Liver diseases and traditional medicine

A large number of natural products with hepatoprotective activity have been reported; for example, for the treatment of ALD, individual plant extracts,[12–14] combinations of extracts,[15–17] and isolated compounds[18,19] have been tested, and have shown a favorable result (Figure 1). In particular, several molecules isolated from plants have also demonstrated their biological potential against different human conditions; however, certain studies have verified its natural origin does not exclude limitations or the possibility of inducing side effects in humans (Figure 1). Such is the case of Saikosaponin D, a compound present in *Bupleuri radix*, which has antitumor and anti-inflammatory activity, but it has the disadvantage that it can be toxic to the liver, brain, and heart even in controlled doses, in addition to altering pharmacokinetics; therefore, its oral bioavailability is controversial.[20] For this reason, nanomedicine-based studies were performed to develop liposomes loaded with Saikosaponin D to overcome its limitations, including its poor solubility and stability, to enhance its hepatoprotective effects,[21] this being only a single example of how nanotechnology can improve the delivery of natural compounds for the treatment of liver diseases (Figure 1).

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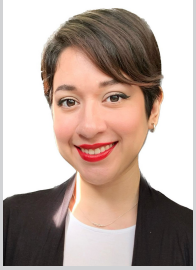

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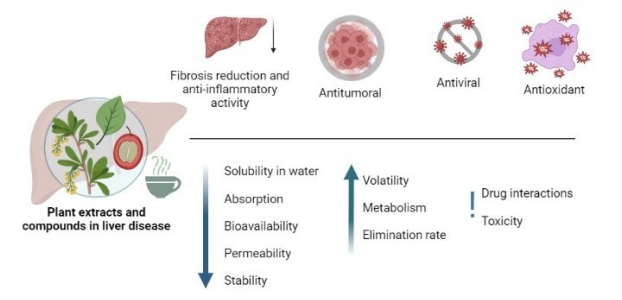

Figure 1. Use of extracts and compounds derived from plants and their limitations in the treatment of liver diseases.

2.1. Controversy on the use of natural products in liver disease

Different types of green tea have been shown to inhibit the conversion of acetaldehyde to acetic acid leading to an increase in acetaldehyde concentration that could aggravate the damage in ALD. The main compounds found in most types of green tea are Gallic Acid, Gallocatechin, Catechin, Chlorogenic Acid, and Epigallocatechin Gallate, which may act synergistically or individually in the prevention of fatty liver disease. In contrast to this preventive effect, the aforementioned tea constituents inhibit the activities of SOD, CAT, and ALDH, which are involved in antioxidant activity,[22] where the need arises to study the adverse effects that the different types of tea may present or to look for alternative dosage forms of their phytoconstituents. On the other hand, an example of a clinical case of ALD of a patient who presented hepatic sinusoidal obstruction syndrome due to the ingestion of *Gynura segetum* for ten days; This plant contains the alkaloid pyrrolizidine (PA), this compound, with 1,2-unsaturated necine base, is known to cause hepatic veno-occlusive disease (HVOD) in humans and animals after acute exposure, which indicates the risk of consuming natural products without medical supervision, since most people are unaware of such effects, and this toxicity may be overlooked in patients with liver disease.[23]

2.2. Antiviral activity

Another major cause of liver disease is viruses. One of the main ones is the hepatitis C virus (HCV), which can lead to complications such as progression to fibrosis. It has been shown that Silymarin, a compound obtained from the seeds of *Silybum marianum*, inhibits the production of pro-inflammatory cytokines secreted by T cells, in addition to its antiviral effect, which could represent a green alternative in the treatment and/or prevention of viral diseases and liver fibrosis.[24] In contrast to the previously mentioned phytoconstituents, Silymarin is not toxic, but it could have limitations in its absorption at the intestinal level due to its low solubility in water; this hydrophobic characteristic highly limits its dose-dependent therapeutic efficacy, because of its bioavailability in the hepatic environment.

On the other hand, the effect of the *Iberis gibraltarica* protein extract was analyzed on HepG2 cells infected with hepatitis C virus (HCV), obtaining that the total protein reduces the RNA of the virus by 20, 48 and 60% when using concentrations of 30, 60 and 90 μg/mL.[25] In addition, two compounds isolated from the methanolic extract of *Dracaena cinnabari* were analyzed, and the results of both showed a dose-dependent decrease in the expression of the HBsAh protein of the hepatitis B virus (HBV) in HepG2.2.2.15 cells. In addition, HBV replication was decreased by 26.7% and 38.2% three days post-treatment. An antiretroviral drug, Lamivuidine, was used as a control, which inhibited only 30.7%, demonstrating the activity of *D. cinnabari* compounds.[26]

The fractions and subfractions obtained from the dichloromethane extract of *Artocarpus heterophyllus* leaves were studied in human hepatoma-derived cells (Huh7it-1); its fraction FR3T3 showed high antiviral activity with an IC_{50} less than 10 μg/mL and low cytotoxic activity. The activity is predominantly in the post-entry stage of the cell by reducing NS3 protein expression and RNA replication. In addition, the presence of terpenoids was reported. In this plant, a compound that showed a synergistic effect when combined with conventional treatments such as Ribavirin, Cyclosporine, and Simeprevir;[27] this synergism makes these terpenoids possible green adjuvants for the treatment of liver diseases. The *in vitro* and *in vivo* anti-hepatitis B activity of Esculetin, a coumarin derived from *Microsorum fortunei*, was tested. Cytotoxicity was tested in normal liver cells, HL-7702, at concentrations of 12.5 to 200 mg/L, showing its low toxicity, and in the HepG2.2.15 cells (hepatocellular carcinoma), an IC_{50} of 217.48 mg/L was found. Likewise, the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis Be antigen (HBeAg) was reduced, compared to the results obtained with lamivudine (3TC), a drug commonly used for the treatment of HBV. The effect on HBV expression in the supernatant of HepG2.2.15 cells was also evaluated after treatment with Esculetin showing strong inhibitory activity. *In vivo*, short-term toxicity was evaluated, showing no effect. The levels of DNA and DHBsAg and DHBeAg antigens of DHBV were quantified in the serum of infected and treated ducks, and after 21 days of treatment, the levels were lower compared to the control, with no liver damage, infiltration, or evident cell damage,[28] thus demonstrating the antiviral activity of this compound of natural origin.

The partition of the methylene chloride extract obtained from the rhizome of *Juncus maritimus* was analyzed for the presence of Dehydrojuncosol, which was tested for its antiviral activity against HCV genotype 2a *in vitro*, using Epigallocatechin Gallate (EGCG) and Boceprevir as a control. It was shown to inhibit replication but not HCV entry. In addition, it has no activity on other viruses of the Flaviviridae family, suggesting that HCV could be targeted by the activity of this compound, with NS5A being the target. It is not toxic against primary human hepatocytes (PHH).[29] From traditional Chinese medicine, Qizhu decoction contains *Phyllanthus urinaria*, *Polygonum cuspidatum*, *Hedoytis diffusa*, *Paeonia lactifora*, *Paeonia veitchii*,

Rhizoma Smilacis Glabrae, and *Salvia miltiorrhiza*. One study analyzed the antiviral activity of the aqueous and ethanol extract *in vivo* and *in vitro*. The results showed that both extracts inhibit hepatic inflammation caused by hepatitis B and even diethylnitrosamine-induced liver cancer by suppressing NF-KB signaling and decreasing TNF-a and IL1B levels and promoting apoptosis. Corilagin and Polydatin were detected in the extracts and have previously been tested for their anti-inflammatory and anticancer activity, now demonstrating their antiviral activity.[30]

As mentioned above, these diseases can lead to complications such as hepatic steatosis, fibrosis, and carcinoma. Traditional medicine has also been tested on these different stages of liver disease. The basement membranes of the hepatocytes are damaged, and the deposition of collagen in the liver increases, specifically type 1 and III, which in this case are the main components of the extracellular matrix in this organ, resulting in fibrosis[31] (Table 1).

2.3. Efficacy of plants to counteract fibrosis

Phyllanthus emblica is a medicinal plant used for the treatment of liver diseases. It has been shown that its aqueous extract can reduce Carbon Tetrachloride (CCl_4)-induced fibrosis *in vivo* by reducing ECM synthesis and accumulation, focal adhesion, and PI3K-Akt signaling pathway to exert anti-fibrosis effects.[32] The medicinal preparation Ger-Gen-Chyn-Lian-Tang has demonstrated its anti-fibrotic activity, which is composed of *Puerarie radix*, *Scutellarie radix*, *Coptidis rhizome*, and *Glycyrrhizae radix*.[16] S-allylmercaptocysteine, a garlic-derived compound, decreases liver injury caused by NAFLD, including collagen formation, by reducing the expression levels of profibrogenic factors and the percentage of collagen distribution in the liver of rats co-treated with the compound. It is therefore considered a hepatoprotective agent.[33]

The saponins contained in the *Panax japonicus* plant have also been studied, and it was observed that in the liver of rats treated with these compounds at low and high doses, they significantly inhibited the production of collagen fibers. They also reviewed the expression of fibrosis markers, observing that TIMP, a-SMA, and Coll were decreased by 51, 57 and 44%, respectively, compared to the control group at a concentration of 100 mg/kg and by 71, 52, and 74% at a concentration of 300 mg/kg, which demonstrates their anti-fibrotic activity.[31] Another plant of the same genus, *Panax notoginseng*, showed the effect of the contained saponins on mice with hepatocyte damage induced by ethanol or a high-fat diet. Specifically, compound K and Ginsenoside Rha, which are the main metabolites of this plant, showed individually or in combination, decreased individually or synergistically the liver damage induced by HFD; additionally, the histological study showed a reversal of fibrosis and cell damage. *In vitro* tests showed that these compounds have the potential to attenuate the expression of fibrotic factors such as TIMP-1, PC-I, and PC-III.[34] Silymarin decreased the degree of liver fibrosis in mice of both sexes which was verified at the histological level and in terms of collagen gene expression and extracellular protein deposition.[35] Moreover, the active compound of Silymarin, Silybin, was shown to inhibit hepatic stellate cell activation and apoptosis in the liver, alleviating hepatic steatosis, fibrosis, and inflammation in mice with DCM-induced NAH[36] (Table 1).

2.4. Efficacy of plants to counteract cirrhosis

Orthosiphom stamineus is used for the treatment of hepatitis. It was shown that there was a reduction in fibrosis and a decrease in hepatic stellate cell infiltration in the liver of rats with thioacetamide (TAA)-induced liver cirrhosis, thus confirming the hepatoprotective effect of the ethanolic extract of *O. stamineus*.[37] On the other hand, liver cirrhosis is a predominant risk factor for hepatocellular carcinoma (HCC). *Oldenlandia diffusa* is a plant used as a cancer treatment; a study demonstrated that the extract of this plant promoted apoptosis, showed antiproliferative activity, and decreased migration capacity in hepatocellular carcinoma cells. *In vivo*, the groups treated with OD and HCC induced by DEN had a greater

Table 1. Efficacy of plants to counteract liver diseases.

Liver disorders	Green Alternative	Type of study	Demonstrated effect	Ref.
	Protein extract from *Iberis gibraltarica*	*In vitro*	Anti-HCV	25
	Isolated compounds from *Dracaena cinnabari*	*In vitro*	Anti-HBV	26
	Extract from *Artocarpus heterophyllus*	*In vitro*	Anti-HCV	27
Viral effect	Esculetin isolated from *Microsorium fortunei*	*In vitro*	Anti-HBV	28
	Dehydrojuncusol extracted from *Juncus maritimus*	*In vitro*	Anti-HCV	29
	Qizhu decoction extracts	*In vivo*	Anti-HBV	30
	Panax japonicus saponins	*In vivo*	Antifibrotic	31
Fibrosis	Silymarin	*In vivo*	Antifibrotic	35
	Silybin	*In vivo*	Antifibrotic	36
Cirrhosis	*Orthosiphon stamineus* extract	*In vivo*	Hepatoprotective	37
	Oldenlandia difusa extract	*In vivo*	Hepatoprotective	38

HCV: Hepatitis C virus, HBV: Hepatitis B virus, Ref: Reference.

survival and, therefore, a greater therapeutic effect than the control group, decreasing the number and size of the tumor, suggesting its potential use as an anticancer drug[38] (Table 1).

2.5. Limits on the use of medicinal plants in the treatment of liver diseases

Although the potential of traditional medicine or the compounds derived from the latter have been proven to have hepatoprotective potential *in vitro* and that they could prevent and/or treat liver disorders, transferring these results from *in vitro* to *in vivo* or to the clinical picture has always been a difficult challenge to overcome; Indeed, this is due to factors such as low bioavailability, solubility, and stability, rapid metabolization, instability in the gastrointestinal tract, lack of organ-specific distribution, and high elimination rate (Figure 1).[39] Echinacoside, a metabolite obtained from *Cistanche tubulosa*, has activity against hepatocellular cancer,[40] but despite these properties, when administered orally, it has poor absorption and low bioavailability.[41,42] Other phytocompounds used for the treatment of liver cancer, for example, the alkaloid Nitide Chloride, also have low water solubility, low bioavailability, and mild toxicity.[43]

It was shown that the increased consumption of alcohol in the preparation called "herbal wine" combined with resveratrol might be a cause of increased liver damage and necrosis since alcohol decreases the absorption of resveratrol by 95%, which decreases its therapeutic effects. It also increases alcohol absorption by 126% and inhibition of the ethanol metabolic pathway.[44] Berberine, an isoquinoline alkaloid obtained from *Berberis vulgaris*,[45] is used to treat NAFLD, as it decreases steatosis and lipid content in the liver by 14% and decreases necrosis in NAFLD and steatosis caused by hepatitis C infection.[46,47] Isoquinoline alkaloid has certain limitations, such as poor water solubility, poor absorption, and poor bioavailability, which limits its applications.[48] In the case of green tea extract, it has been associated with hepatotoxicity since under specific conditions such as fasting, high doses, and repeated administrations, the concentrations of catechins in plasma increase considerably, contrary to what happens when it is consumed with food or at low doses,[49] which implies saturation of the elimination mechanisms, occurring with variability of intake amounts from 140 mg to 1000 mg per day. Even so, there are interindividual variations due to possible genetic factors.[50]

3. Green nanomedicine in the treatment of liver diseases

In liver diseases, there is a shortage of drugs that allow limiting their progression to a chronic condition. Concerning NAFLD, ALD, and viral hepatitis, due to the complexity of their pathophysiology and the different levels of evolution of the disease, developing a drug for their treatment remains a major challenge for modern pharmacology.[5] At present, the therapeutic approach for the control of this condition is based on lifestyle changes, the use of insulin-regulating drugs to reduce weight (number of adipocytes), and the consumption of natural compounds such as antioxidants to prevent and/or limit the progression of this condition.[6]

On the other hand, it is known that the main limitation in the cure of liver diseases is directly related to the inability of potentially active molecules to reach their pharmacological target, i.e., to reach the liver at the desired therapeutic concentration or therapeutic bioaccumulation; additionally, there is a shortage of drugs specifically designed for release in liver tissue or liver cell type depending on the level of development of the disease.[51] However, several studies consider nanoparticles (Figure 2) as a promise to potentiate the bioaccumulation of actives in the liver due to their physicochemical characteristics such as their size, surface charge, drug protection against degradation, the possibility of molding them to suit the biochemical conditions imposed by the disease, their capacity to improve the amount of encapsulated drug, and to encapsulate drugs of different polarities or nature;[5,7,9,11] These characteristics together (Figure 2) allow for increased intestinal absorption, increased bioavailability of the active ingredient in the liver and limited side effects due to *in situ* accumulation of the drug.

3.1. Generality of the liver environment

It is evident that the changes in the clinical cases imposed by the evolution of liver diseases through the evolutionary deterioration of the liver constitute an important biological barrier in the treatment of these diseases, given that each clinical case presents a different microenvironment; therefore, understanding the changes that occur at the cellular and molecular level in each state of liver diseases could represent

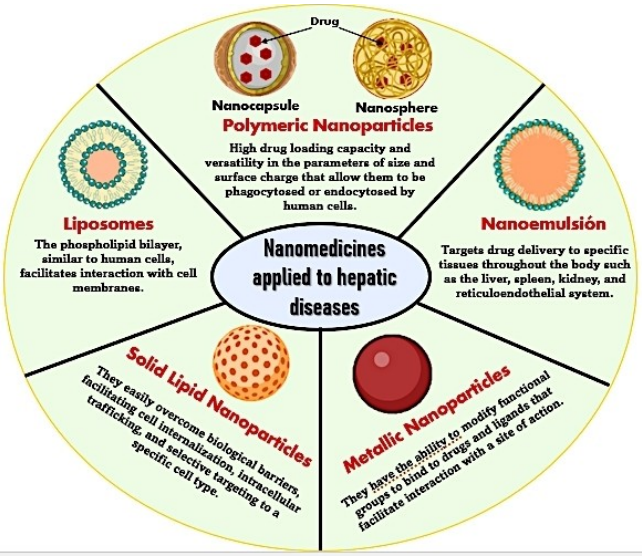

Figure 2. Types of nanoparticles and their physicochemical characteristics.

an essential key for the adequate design of the dosage forms (i.e., nanoparticles) of drugs or actives, and thus facilitate their bio-accumulation, overcoming the biological barriers generated by the clinical cases of the disease. From this point of view, the liver, in a normal physiological state, is specifically composed of hepatocytes, sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells[52] (Figures 3 and 4A); sinusoidal endothelial fenestration (50 to 200 nm) being the main filter for the transfer of plasmatic particles to hepatocytes.[53] Therefore, only nanoparticles smaller than 200 nm can reach the Disse space and, consequently, the stellate cells and hepatocytes (Figure 4).

3.2. Efficacy of green nanoparticles to counteract fibrosis

Fibrosis is commonly manifested by hepatic lesions and inflammations. Indeed, lesions are caused by the excessive production of collagens, while inflammations are induced by the synthesis of chemokines that promote the chemotaxis of inflammatory and tumor cells; it should be noted that the synthesis of collagens and chemokines takes place in the hepatic stellate cells, through their activation.[9] Since the activation of stellate cells is the main event in inducing fibrosis, as well as tumor metastasis and hepatic carcinoma, finding drugs that inhibit this cell activation and formulating them appropriately could allow curing and/or limiting the progression of fibrosis to more serious clinical cases (cirrhosis/carcinoma). Indeed, several studies have shown that phytochemicals have anti-inflammatory, hepatoprotective *in vitro*, hepatoregenerating, anti-inflammatory, and other activities;[54–56] this means that they can help in the cure and/or prevention of fibrosis. However, transferring these results in *in vivo* models to the clinical setting has been an extremely high challenge, due to therapeutic failure, undoubtedly due to the low accumulation or pharmacological bioavailability of the active molecule in the desired site, to the difficulty that these phytoconstituents have in overcoming the biological barrier of the fibrotic environment; therefore, the adequate design of the pharmacological dosage form is the second challenge to transfer the results obtained *in vitro* and *in vivo* to the clinical setting.

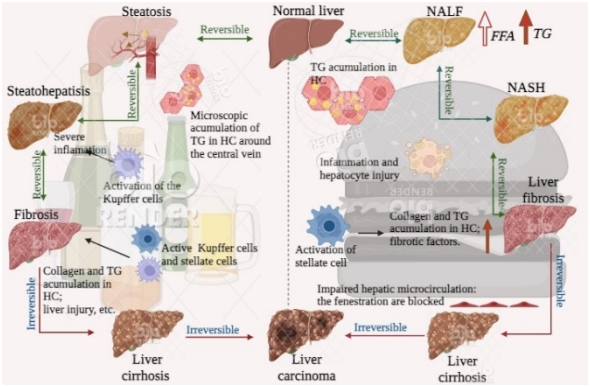

Figure 3. Stages of development of liver diseases induced by the consumption of alcohol and fast food (initially induced by the increase of triglycerides followed by the activation of stellate and Kupffer cells; finally, due to cirrhosis, the fenestrations close, preventing the passage of plasmic particles in the space of Disse). HC = Hepatic Cell. TG = Triglyceride. FFA: Free Fatty acid. NALF = Non-Alcoholic Fatty liver. NASH = Non-Alcoholic Steatohepatitis.

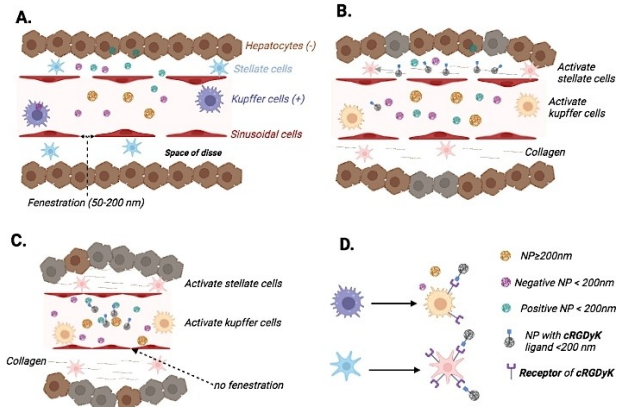

Figure 4. Characteristics of nanoparticles (NP) and their biodistribution in the hepatic microenvironment in different clinical conditions. A) normal liver; B) fibrotic liver; C) cirrhosis liver; D) NP with ligands and their drug target.

According to Yanping Li et al.,[57] the main characteristic of activated stellate cells (aHSC) is the overexpression of αvβ3 integrins; therefore, the design of nanoparticles with the capacity to reach this target would represent the main challenge to inhibit fibrosis and limit its progression into more complex clinical cases. It is important to insist on the fact that this author proved that liposomal nanoparticles with cyclic peptides (cRGDyK) as ligands (Figure 4D) have a high affinity for αvβ3 and easy internalization by aHSCs. On the other hand, Abdullah and co-workers found that silymarin-loaded chitosan nanoparticles limit the expression of major fibrosis mediators such as TGFβR1, COL3A1, TGFβR2 and increase hepatic expression of protective miRNAs (miR-22, miR-29c and miR-219a).[58] In a similar work, Yousni et al. proved that Silymarin encapsulated in Eudragit RS100 has anti-fibrotic activity and could inhibit fibrogenesis. Indeed, in their study, they obtained a spherical particle size of 632.28 ± 12.15 nm, with a prolonged release of the active ingredient; The results showed that Eudragit RS100 nanoparticles decreased serum tumor necrosis factor-α (TNF-α), serum transforming growth factor-β1 (TGF-β1), hepatic hydroxyproline, hepatic expression of tissue inhibitor metalloproteinase-1 (TIMP-1) and cytokeratin-19 (CK-19); Conversely, increased hepatic hepatocyte growth factor (HGF) and hepatic expression of matrix metalloproteinase-2 (MMP-2) and increased MMP-2/TIMP-1 ratio at the mRNA level were observed.[59] It should be noted that due to the size of their nanoparticles, it is evident that these nanoparticles cannot reach the Disse space; also, due to the nature of the polymer employed (PH-dependent), these nanovehicles considerably increased the absorption of Silymarin at the intestinal level, thus favoring its bioaccumulation in the liver; therefore, an improvement in the physicochemical characteristics of these nanoparticles could optimize the anti-fibrotic effect *in vivo.* Concerning curcumin, several studies have shown that it may have anti-fibrotic and hepatoprotective activity, among others.[60–62] According to Elzoheiry,[63] *in silico* molecular docking revealed that curcumin can inhibit fibrosis-mediating proteins

such as PDGF, PDGFRB, TIMP-1, and TLR-9 by direct binding; he also proved in this same study that silver nanoparticles with curcumin and coated with chitosan increased the hepatic anti-inflammatory effect of curcumin, as well as reversed the fibrotic effect induced by CCl_4 in an *in vivo* study. In a similar study, curcumin formulated in polymeric nanoparticles was found to enhance antioxidant levels in the liver, inhibit profibrogenic transcripts associated with activated myofibroblasts and induce stellate cell apoptosis *in vitro*.[64] In contrast to the previously cited works, the authors did not focus on the impact that the physicochemical characteristics of the nanoparticles obtained could have; therefore, considering them could represent an important advance in the characteristics that nanocarriers should have to improve the therapeutic efficacy and in situ bioavailability of the drug, as well as to facilitate the transfer of the results obtained *in vitro* and *in vivo* to clinical cases.[11]

3.3. Cirrhosis/liver carcinoma

Several investigators report cirrhosis and hepatic carcinoma as chronic, irreversible[8] clinical cases due to the different physiological barriers established in the liver, which limit and/or prevent free drugs, as well as those formulated in nanoparticles, from reaching their pharmacological target (stellate cells, hepatocytes); it should be noted that in these clinical cases, the hepatic cells with greater therapeutic accessibility are the Kupffer cells and sinusoidal cells (Figure 4B). The major challenge in cirrhosis is the blockage of fenestrations between sinusoidal cells, which highly limits the crossing of plasma components to the Disse space and consequently to hepatocytes and stellate cells (Figure 4C). Previous studies report that the main causes of liver disease complications are activation of stellate and Kupffer cells, which are related to the healing phenomena of hepatic inflammation, respectively; however, activation or destruction of sinusoidal cells is not reported, so that closure of fenestrations would be related to liver compression by solidification of or destruction of hepatocytes highly loaded by collagen. Indeed, we hypothesize that the reduction of the number of sinusoidal cells by inducing apoptosis, accompanied by hepatoregenerating, anti-inflammatory drugs, specifically targeting stellate, Kupffer cells, could represent a solution in the cure of cirrhosis. In addition, to this, a treatment based on potential antioxidants to fulfill the function of hepatoprotection, capturing the free radicals present in the hepatic microenvironment, could be added. This would involve the use of active ingredients formulated in different forms or different types of nanoparticles (Figure 4D) with different physicochemical characteristics and a controlled release at different sites.

3.4. Opinion

Emphasizing the impact of the physicochemical characteristics of nanoparticles, there is a scarcity of results discussing it. Indeed, most studies discuss the size of the particles, generally omitting the impact that their electrostatic charge could have on the pharmacological orientation of the nanoparticles towards the target liver cells, which could increase the pharmacokinetics of the nanoformulation. This lack of results could be related to the limited knowledge of the surface characteristics of the cells involved in the clinical cases of liver diseases, as well as the evolutionary change of the latter induced by chemical and structural changes in the hepatic microenvironment. In specific cases, it is known that Kupffer cells have a positive and negative surface charge (Figure 3), respectively; however, once activated, the electrostatic charge may have been unknown, but rather the receptors they have on their surface where the dosage forms using precision nanoparticles allow a specific orientation of the latter towards the target cells (stellate cells).

Concerning the size of nanoparticles, it should be noted that they have been the most studied physical characteristic of nanoparticles for the controlled release of activity in the liver, for their bioaccumulation in the desired target site (space of Disse), etc. However, the physiological changes of the hepatic microenvironment in fibrosis, through the reduction of hepatic fenestrations, could represent an important limitation in the accumulation of the active at pharmacological doses in the Disse space since smaller nanoparticles would be needed (Figure 2). In this respect, finding better nanoparticle systems would be ideal for improving pharmacodynamics and pharmacokinetics, allowing the therapeutic bioaccumulation of the drug in the Disse space, such as in hepatocytes and stellate cells. Additionally, it is important to mention that liver diseases, regardless of the evolutionary state in which it is, uncouple a set of metabolic and cellular events so that a solution to reverse them quickly would be to deliver nanoparticles of different characteristics oriented toward a specific target; hypothetically, without the need to study the synergisms between the active green nanoencapsulated, since the nanoparticles would be designed for intracellular release.

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Homage to Mrs. **Yango Augustine**, who fought for months against the hepatic complications.

Conflict of Interests

The authors declare no conflict of interest.

Keywords: green molecule · green nanomedicine · liver diseases · specific drug delivery · traditional medicine

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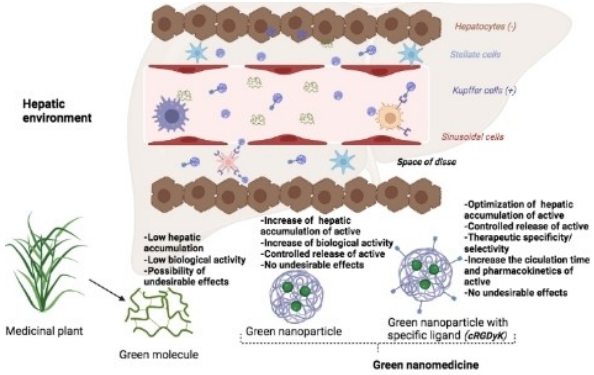

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1 – 10

Green Alternatives in Treatment of Liver Diseases: the Challenges of Traditional Medicine and Green Nanomedicine

Green alternatives in the treatment of liver diseases: the challenges of traditional medicine and green nanomedicine

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