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Protective effects of curcumin against ischemia-reperfusion injury in the liver

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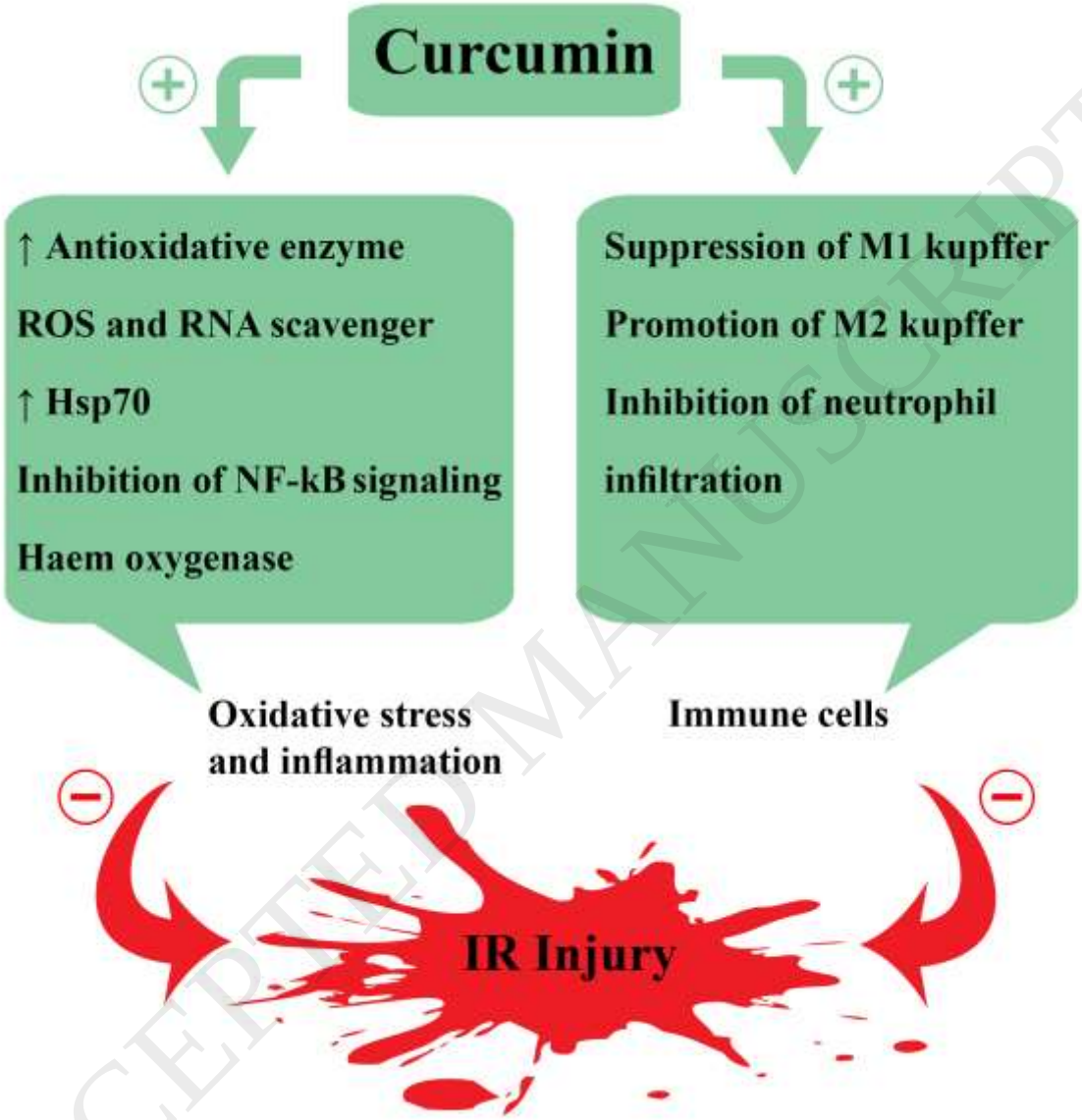
Graphical abstract



Abstract

Liver ischemia/reperfusion (I/R) injury is a major complication of hepatic surgery and transplantation. It is one of the leading causes of morbidity and mortality because of post-surgery

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hepatic dysfunction. Several studies have suggested different mechanisms are involved in the pathogenesis of I/R injury in the liver that includes oxidative stress, inflammation, mitochondria dysfunction, liver Kupffer cells (KCs) activation, vascular cell adhesion molecule overexpression, and facilitation of polymorphonuclear neutrophil injury. Curcumin is a natural product extracted from *Curcuma longa* that is known to suppress these pathways and as a result reduces liver ischemia-reperfusion injury. This paper gives an overview of the protective effects of curcumin against I/R injury in the liver and discusses the studies that have linked biological functions of curcumin with liver I/R injury improvement.

Keywords: Ischemia-reperfusion injury; curcumin; Liver; transplantation.

1. Introduction

In liver resection and liver transplantation when the blood flow is blocked and then restored, which are called ischemia and reperfusion phases, intense hepatocyte damage occurs. Ischemia/reperfusion (I/R) injury in the liver causes liver nonfunctional or functional failure after surgery [1]. The detailed mechanisms of liver I/R injury are complex and at the moment unclear, but some studies have shown that several signaling mechanisms are involved in the pathophysiology of liver I/R injury including inflammation, oxidative stress, mitochondria-mediated mechanisms, Kupffer cells activation, apoptosis and increased proinflammatory cytokine signaling. These maladaptive processes begin in the ischemic phase and then intensify in the reperfusion phase [2, 3]. Several studies have shown that phytocompounds such as cyanidin 3-O-beta-d-glucoside (C3G) [4, 5], *Inula racemosa* root [6] [7], hydroxytyrosol [8], pycnogenol [9] and curcumin [10] have therapeutic effects in liver I/R injury. Previous investigations have

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indicated that curcumin is one of the compounds that can be effective in the treatment of liver I/R injury.

Curcumin, a yellow extract from the Indian spice turmeric, has been reported to have many properties including anti-inflammatory [11, 12], antioxidant [13-16], anticarcinogenic [17-19], antimicrobial [20], analgesic [21], pulmonoprotective [22], lipid-modifying [23-25] and hepatoprotective [26-28] properties. Several lines of experimental evidence have shown that curcumin has protective effects against I/R injury in the liver through its antioxidant and anti-inflammatory effects [2, 29, 30]. The aim of the present review was to explore the protective effects of curcumin against I/R injury in the liver.

2. I/R injury in liver

Liver I/R injury results from the restoration of the blood supply following its attenuation or cessation. Together, these lead to the impairment of oxygen dependent cells within the liver and subsequent organ dysfunction [31]. Liver I/R injury is a major clinical problem in liver transplantation and resection surgery that leads to microcirculatory failure and consequently cell death (based on ATP availability, necrosis or apoptosis) with a considerable effect on liver function prognosis [32-35]. Liver I/R injury can lead to the higher incidence of both chronic and acute rejection and causes up to 10% of early organ failure. However, an effective method its prevention and treatment has not yet been identified, which is important as reducing the negative effects of I/R injury can increase the success of transplantation in patients [36]. Two major types of liver I/R injury can be distinguished (Figure 1). The ‘warm’ I/R injury which occurs during prolonged surgical liver resection through clamping of blood perfusion, or various forms of shock, trauma,

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heart failure, respiratory failure, hemorrhage, and sepsis [37, 38]. In contrast, 'cold' I/R injury follows liver transplantation that occurs because of the ex vivo preservation to keep the donor organ cold [39]. Furthermore, the primary cellular targets of the two types of injury are different. For example, 'warm' I/R injury is initiated by hepatocellular damage, whilst 'cold' is initiated by damage to hepatic sinusoidal endothelial cells and disruption of the microcirculation [40]. In clinical liver transplantation, 5% of grafts failed without a specific reason. The occurrence of initial graft nonfunction is dependent on the time of cold storage and so is related to injury associated with cold ischemia and warm reperfusion [41]. Ischemic preconditioning decreases hepatic enzyme release and mortality after warm ischemia and reperfusion in the liver [42]. However, there are conflicting results about preconditioning against cold storage-reperfusion injury. One study showed that ischemic preconditioning increased graft survival after liver transplantation in animals [43], while another study demonstrated that ischemic preconditioning made livers more sensitive to storage-reperfusion injury [44]. It has been shown that the cold preservation injury favors an early and massive T-cell influx into ischemic liver grafts [45].

No definite treatment is known for liver I/R injury; however, limiting the duration of cold preservation and re-warming during surgery remain primary strategies to decrease the harmful effects of liver I/R injury. Cold perfusion also limits the duration of organ storage because it might enhance vascular resistance and damage to sinusoidal endothelium and endoplasmic reticulum [46]. It has been shown that tissue damage in liver I/R injury happens in early and late phases. The early phase is caused by Kupffer cells (KCs), hepatocytes, and sinusoidal endothelial cells (SECs) in the first 6 hours following reperfusion. It is believed that this phase is a result of rapid changes in the redox state of liver tissue [47, 48], while the late phase is through infiltration of leukocytes into the liver tissue and the consequent production of chemokines and cytokines [49]. The degree

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of I/R injury severity depends not only on the duration and the methods used in hepatic ischemia and reperfusion induction, but also on the background of the liver condition [50]. Some measurements are normally performed as indicators of hepatic I/R injury that include elevated levels of hepatic enzymes, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) along with histological evaluation based on the Suzuki classification [51, 52]. A complex molecular mechanism and cellular interactions have been implicated in liver I/R injury that includes formation and scavenging of reactive oxygen species (ROS), intracellular calcium overload, anaerobic metabolism, mitochondria, liver Kupffer cell (KCs) activation, vascular cell adhesion molecule overexpression, released pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β, chemokines and neutrophil influx [53, 54]. Furthermore, studies have identified a series of differentially expressed genes that mediate the physiological and biochemical events triggered by hepatic I/R injury. Toll-like receptor 4 (TLR4) is one of the genes that is overexpressed in liver transplantation and its down-regulation alleviated liver I/R injury. Recent studies on organ transplantation surgery have identified the regulatory role of microRNAs in I/R injury. For example, 40 differentially expressed microRNAs related to proinflammatory processes were detected in I/R injury post-liver transplantation. miR-223 was up-regulated while miR-146a was down-regulated in liver I/R injury [55, 56].

3. Animal models of liver I/R injury

Animal models used for the induction of cold and warm hepatic I/R injury have been reviewed recently [35], highlighting that they are valuable tools for understanding the physiopathology of hepatic I/R injury, and helping to discover novel therapeutic targets and drugs. Various species have been used as animal models for hepatic I/R injury from mice to pigs in a continuum from

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feasibility to human translatability [57]. Four models of global normothermic liver ischemia including the Pringle maneuver, splenocaval shunt, portojugular shunt, and spleen transposition along with partial liver ischemia and different liver transplantation models optimized with static organ preservation or machine perfusion (Normothermic, hypothermic and subnormothermic) are commonly used models of I/R injury [57]. Various strategies have been investigated to reduce or prevent hepatic I/R injury in animal studies, including a pharmacological approach, preservation solutions, gene therapy and surgical strategies [35]. There are some additional points that should be considered. Firstly, the type, extent and time of ischemia are among the factors that alter the pathophysiological mechanisms of hepatic I/R injury. For example, hypothermic organ perfusion is based on decreasing cellular metabolism by lowering the temperature of the organ. Hypothermic oxygenated perfusion of pig liver grafts can give a survival benefit that is related to a reduction of necrosis and platelet adhesion, improvement of bile flow and the return to usual levels of ATP and glutathione [58]. Secondly, short (60 min) and long (120-180 min) periods of warm ischemia lead to reversible and irreversible cell damage, respectively [59]. There is a dependency between the underlying mechanism of hepatic I/R injury and the experimental model used, so the therapeutic strategy should be adopted to these differences between animal models, and need to be taken in to account when translating the results of the animal studies into clinical practice [57].

4. Curcumin as therapeutic strategy

Curcuma longa, customarily named as turmeric belongs to the ginger family. A natural polyphenol found in the roots of *Curcuma longa* is curcumin. The molecule of curcumin is light yellow and it is used as natural food color [60]. Curcumin is used as a spice, food preservative, coloring agent, and in Ayurvedic medicine for the treatment of different diseases [61]. Constituents isolated from

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the rhizomes of *Curcuma longa* include demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and Curcumin, the latter of which is the major compound found [62, 63]. Commercially available extracts of curcumin may consist of 77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin [64]. The chemical structure of curcumin was discovered in 1910 as 1,6-heptadiene-3,5-dione-1,7-bis (4-hydroxy-3-methoxyphenyl)-(1E, 6E) or diferuloylmethane [65]. Because of the low absorption of curcumin due to its fast metabolism in the intestinal wall and liver following oral administration, Guido Shoba et al., showed that the consumption of curcumin with piperine increased the bioavailability of curcumin by 20-fold [66, 67]. Also, a micellar surfactant and phospholipid complex has been noted to increase absorption 9-fold and 3.4-fold, respectively [68, 69]. Curcumin has numerous mechanisms and cellular targets through which it exerts its biological effects. These targets include growth factor receptors, transcription factors, protein kinases, adhesion molecules, apoptosis-related proteins, inflammatory cytokines, enzymes like ATPase, cyclooxygenase-2 and matrix metalloproteinase [70]. Curcumin has been reported to possess a wide range of biological activities including antioxidant activities [71], antivenom activity [72], antimicrobial effects [20], anti-HIV activity [73], anti-tumour activity [74], anti-inflammatory activity [75], antiprotozoal activity [76], nephroprotective activity [77], antirheumatic activity [78], affect skin diseases [79] and therapeutic activity for the treatment of hepatic disorders (Figure 2) [2].

5.1 Curcumin against I/R injury in liver

There is a large body of evidence mainly derived from animal studies showing that curcumin protects against ischemia/reperfusion injury in different organs including brain, heart, kidney, intestine, ovary, testis and also liver [57, 80-87].

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The possible protective effect of curcumin for I/R hepatic injury has been investigated with the help of different models of ischemic reperfusion, reduced-size liver warm ischemia and reperfusion. 1 or 5 mg/kg of curcumin, which was injected through left femoral vein, was able to significantly improve the morphological damage due to acute liver lesions following intestinal I/R manifested by bleeding, neutrophil infiltration, and edema in the liver tissues in an established one hour ischemia-two hour reperfusion in male Sprague-Dawley rats [88]. Curcumin was administrated orally in the majority of these studies and intra peritonealy or intravenously in some limited cases. In a study that was performed on a rat model of hepatic I/R injury induced by liver blood flow arrest, biochemical (Total antioxidant capacity (TAC) and malondialdehyde (MDA)) analyses along with histopathological evaluation revealed that administration of 200 mg/kg of orally gavaged curcumin, 15 minutes before ischemia induction was not able to ameliorate the hepatic IR injury [89]. Conversely, there is a considerable amount of experimental data indicating the effectiveness of curcumin on ameliorating hepatic IR injury (Table 1). It has been reported that the protective effects of curcumin against hepatic warm I/R injury are mainly due to the effect on antioxidant enzymes and heat shock proteins. Specifically, pre-treatment injection of 50 mg/kg curcumin, 30 minutes before ischemia lessens the apoptosis rate, activity of inducible nitric oxide synthase (iNOS), levels of malondialdehyde(MDA), NO2-, NO3- and myeloperoxidase (MPO) in liver tissue, while increasing the activity of catalase (CAT) and superoxidase dismutase (SOD), Hsp70 expression and the 7 days post reperfusion survival rate [90]. In another study, curcumin in combination with simvastatin, N-acetylcysteine, erythropoietin, pentoxyphylline, melatonin, glycine, and methylprednisolone was evaluated for the reduction of postischemic reperfusion that was performed in histidine-tryptophan-ketoglutarate solution with 37°C Krebs Henseleit bicarbonate buffer 24 h after cold I/R storage of the isolated rat livers, and it was shown that this

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combination successfully eliminated the inflammatory response and entirely prevented the parenchymal injury symptoms. As hepatic I/R injury is a multifaceted phenomenon, multidrug donor preconditioning with a relatively safe compound such as curcumin seems to be a promising approach as long-term therapy (more than 10 days) is necessary for an optimum outcome [91].

In addition to the research detailed above that were focused on donor preconditioning with curcumin, other investigations that target the effect of curcumin in liver preservation phase have been undertaken. In one of these studies, comparing the preservative effects of flushing Sprague-Dawley rat livers with different solutions including Euro-Collins solution (EC), phosphate buffer saline (PBS), University of Wisconsin solution (UW)] with or without curcumin (25-200 microM) and storing them in 4° C for 24-48 hours showed that 100μM curcumin was the optimal concentration and that curcumin successfully enhanced the time and quality of liver preservation in PBS, EC and UW solutions [92]. In another study, it was shown that curcumin enhanced Euro-Collins (EC) solution was equivalent to University of Wisconsin (UW) solution in an ex vivo model of liver preservation; in this comparison, Sprague-Dawley rat livers were flushed with preservation solutions of UW or EC with or without 100 μM curcumin and then underwent oxygenated perfusion after 24 hours of cold storage at 4°C [93].

5.1.1 Cellular and molecular mechanisms underlying protective effect of curcumin against I/R injury in liver

Understanding the mechanisms of hepatic I/R injury is critical for the development of preventive strategies. The pathophysiology of hepatic I/R injury results from different mechanisms including inflammation that results from cellular damage, oxidative stress, pro- and anti- inflammatory mediators, and adhesion molecules (Figure 3) [52]. Several molecular mechanisms underlying the

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protective effects of curcumin against hepatic I/R injury have been identified that are described below in detail.

5.1.1.1 The Effect of Curcumin on Inflammation, Nitrosative and Oxidative Stress

Antioxidants ameliorate hepatic reperfusion injury by intracellular detoxification of reactive oxygen species that are generated intra or extracellularly [94-96]. The initiator of liver injury is ROS production attributed to a shift from anaerobic metabolism during the ischemic phase into aerobic metabolism that occurs in the reperfusion phase. In addition to hepatocytes, other sources of ROS production in early, mid and late stages of I/R injury include, Kupffer cells, natural killer T-cells and neutrophils. ROS production leads to both direct cellular injury and activation of a molecular mediator cascade, which leads to microcirculatory dysfunction, hepatocyte and sinusoidal endothelial cell injury in the form of apoptosis or necrosis. Liver injury not only increases the oxygen radicals, but also induces nitrosative stress and TNF production [30]. It was reported that a substantial portion of the protective role that antioxidants play against the I/R injury in liver and other organs such as heart, intestine and kidney was due to their ROS suppression effects [97]. Curcumin increases the expression and activity of antioxidant enzymes [98, 99]. It has also been reported from *in vitro* studies that curcumin acts as an efficient ROS and reactive nitrogen species (RNS) scavenger with mediatory effects on a variety of transcription factors, growth factors, inflammatory cytokines and protein kinases (Figure 4) [99]. iNOS and endothelial nitric oxide synthase (eNOS) produce two different types of NO free radical that act in diametrically opposite ways. For example, iNOS damages liver tissue both directly and indirectly through upregulation of the inflammatory responses, whilst eNOS is beneficial for the liver [100, 101]. In spite of post hepatic warm I/R injury overexpression of iNOS [90], the consequence of

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competition for L-arginine substrate with arginase enzyme [102] together with hypoxia induced NADPH deficiency does not result in NO accumulation at the early stage of hepatic warm I/R injury, but rather in the later stages in association with other factors, such as neutrophils and activated Kupffer cells [90]. It was reported that orally administrated curcumin in lipopolysaccharide (LPS)-injected mice could reduce the iNOS mRNA level by 50%-70% [103]. In addition, it has been reported that NO production in activated macrophages could be inhibited effectively through suppression of iNOS expression at the level of both mRNA and protein with low concentrations of curcumin [104, 105]. It was also reported that pretreatment with curcumin decreased the post reperfusion injury iNOS activity in a rat model, whilst having no effect on the eNOS activity in liver tissues: a substantial decrease in the nitrate/nitrite ratio was also observed [106].

5.1.1.2 Curcumin-induced expression of Hsp70

Heat-shock protein chaperones are generated as critical elements of cell protective mechanisms against stress [107, 108]. Among them, Hsp70 is known to have a well-established role in organ protection against I/R injury. Induced overexpression of Hsp70 ameliorates the I/R injury by suppressing the iNOS expression and consequently decreasing NO production or playing a mediatory role in iNOS inhibition [109, 110]. In addition, through overexpression of Hsp70, cells can protect themselves from death and apoptosis [111, 112]. Curcumin potently stimulates the expression of Hsp70 (Fig 4) in stressed cells [113, 114]. In a rat model of warm hepatic I/R injury it was observed that Hsp70 overexpression in liver tissue occurred both at the mRNA and protein levels following the pretreatment with curcumin, and with the concomitant inhibition of I/R induced iNOS activity and apoptosis [106].

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5.1.1.3 Curcumin mediates the NF-κB signaling pathway

It has been shown that TLR4/nuclear factor (NF)-kB pathway can regulate the hepatic I/R injury by induction of an inflammatory response [115, 116]. In one study, TLR4/NF-κB was identified as a major therapeutic target of curcumin in reducing hepatic I/R injury [10]. This group proposed that this protective effect of curcumin in a rat model was through inhibiting the nuclear translocation of NF-κB (Figure 4), which leads to a reduction in the levels of alanine aminotransferase (ALT), aspartate alanine aminotransferase (AST), and inflammatory cytokines (TNF-α, IL-6, and IL-1β) in hepatic I/R-injured rats. In a conformational step, western blot analysis showed that curcumin inhibits the TLR4/NF-kB pathway in hepatic I/R-injured rats [10]. Another group also suggested that inhibition of NF-κB pathway by curcumin attenuates hepatic injuries induced by intestinal I/R that can be attributed to its antioxidative and anti-inflammatory effects shown by decreases in serum AST and ALT levels, increased SOD activity, decreased IL-6 and TNF-α levels along with decreased MPO activity and NF-κB expression compared to the non-treated I/R group [88]. ICAM-1 is one of the immunoglobulin superfamily that is involved in adhesion and emigration of activated leukocytes in venules. Studies have shown that ICAM-1 is upregulated following hepatic I/R injury that was under NF-kB activation. Curcumin has been shown to decrease liver overexpression of ICAM-1 following hepatic I/R injury, suggesting that curcumin could suppress leukocyte adhesion to endothelia [88].

5.1.1.4 Curcumin as immune mediator

Kupffer cells are a group of hepatic macrophages that are the largest population of tissue-resident macrophages in the body. They play a pivotal role in the immune response. In the healthy persons,

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Kupffer cells phagocytize majority of the pathogens such as microorganisms and their products. They are also responsible for the clearance of the other factors including aged erythrocytes, cell debris, tumor cells, and immune complexes [117, 118]. In the diseased state, pro-inflammatory cytokines such as TNF-α, IL-6, IL-12, IL-1β, and inducible nitric oxide (NO) synthase are secreted by a group of Kupffer cells, while another type may work contrarily to increase the expression of anti-inflammatory mediators [119]. Studies have revealed that macrophages can attenuate liver I/R injury by decreasing local neutrophil accumulation, pro-inflammatory factor expression and increasing the level of IL-10 [120, 121]. Primed Kupffer cells result from stimulation by complement factors. Kupffer cells are remarkably plastic cells that express a range of phenotype polarization based on their local environment, with M1 (pro-inflammatory) and M2 (anti-inflammatory) features as the two extremes of this spectrum. It has been shown that one of the mechanisms of hepatic I/R injury amelioration by curcumin is suppression of M1 Kupffer cells and promotion of M2 polarization in these macrophages [122]. In addition, It has been shown that the curcumin derived improvement in hepatic I/R injury was a result of the functional inhibition of Kupffer cells by peroxisome proliferator-activated receptor γ (PPARγ) activation, which in turn down-regulated NF-κB signaling pathway [122]. The other important immune cells in I/R injury are neutrophils that are also inducers of oxidative stress and proinflammatory cytokines, chemokines and activated complement factors [123]. Curcumin was shown to inhibit neutrophil infiltration (Figures 4 and 5) [124].

6. Some other possible mechanisms activated by curcumin

It has been shown that haemoxygenase could ameliorate hepatic I/R injury by reducing cytokine release from Kupffer cells resulting in a decrease in neutrophil recruitment. Theoretically,

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curcumin as a haemoxygenase inducer (Figure 4) may exert at least part of its hepatic I/R reperfusion improvement effects through this mechanism, though this requires confirmation [125]. The pharmacological intervention with curcumin mechanistically ameliorates different stages of the complex pathophysiology of hepatic I/R injury. Preventing the conversion of sub-lethal ischemia, which is induced by cell injury, to lethal ischemia through suppressing the ROS generation in the reperfusion phase, is one of the critical mechanisms by which curcumin has its effects. In addition, reducing the recruitment and activation of Kupffer cells and neutrophils whilst suppressing proinflammatory mediators could be another aspect of hepatic I/R injury inhibition that is observed following curcumin administration. Thus, all these indicate the multidimensional effects of curcumin by targeting different steps and mediators of hepatic I/R injury.

7. Future perspectives of the use of curcumin in human liver transplantation

The development of an effective protective agent to reduce hepatic R/I injury is the major goal that would have major clinical implications in liver transplantation practice. Animal models are important for the investigation of these agents, but clinical trials in subjects are the ultimate way to show efficacy. Curcumin may have utility in liver transplantation, firstly, as a highly available, safe and low cost antioxidant and secondly, as a potential therapeutic agent for non-alcoholic fatty disease (NAFLD), a common liver disease that may lead to cirrhosis. NAFLD is characterized by narrowing of the sinusoidal lumen due to accumulation of fat in hepatocytes, impaired tissue microcirculation and oxygen transfer [126, 127] [128], which contributes to increased resistance against I/R injury. A number of herbal compounds with antioxidant activity have shown efficacy at ameliorating hepatic I/R injury in animal models, but high dose and prolonged pretreatment is necessary for these herbal compound to be effective [129]. There are currently no clinical trials

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on either the safety or efficacy of curcumin to ameliorate hepatic I/R injury, or to improve liver transplantation success in human subjects, in part due to the low bioavailability of curcumin, which may be addressed through slow release nanoformulations.

8. Nanodelivery of curcumin in I/R injury

The bioavailability of curcumin has been limited by poor absorption, rapid metabolism, and prompt systemic elimination [130]. During the past decade, various methods have been investigated to overcome these limitations including different delivery platforms and synthesizing curcumin analogs [131, 132]. Nanodelivery of curcumin is one of those approaches that may have merit in the prevention or amelioration of I/R injury. Nanocurcumin ameliorated the development of ovarian I/R injury in female wistar rats following intraperitoneal injections. In this regard, only mild ovarian hemorrhage with minimal inflammation was observed in curcumin treated rats compared to the severe hemorrhage, vascular congestion, degenerative and necrotic changes seen in untreated rats. Biochemical assessment showed that curcumin decreased nitric oxide synthase (NOS) activity, malondialdehyde, myeloperoxidase and 8-hydroxy-2 deoxyguanine (a DNA damage product) [133].

Others have evaluated the effect of 8-day administration (5 days pre and 3 days post event) of solid lipid nanoparticles of curcumin (25 and 50 mg/kg) in cerebral ischemia injured rats. Confocal microscopy confirmed the presence of these nanoparticles in brain tissue sections. There was a remarkable increase in neurological scores and cognition, whilst biochemically there was a decrease in lipid peroxidation, nitrite and acetylcholinesterase. Administration of curcumin nanoparticles has been associated with restoration of sham control levels of superoxide dismutase, glutathione, catalase, and the mitochondrial complex [134]. Renal I/R induced in C57/B6 mice

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was ameliorated by liposomal curcumin composed of egg phosphatidylcholine and prepared by the thin film lipid method, reflected an improved serum urea and creatinine, reduced tubular injury, decreased apoptosis, reduced neutrophil infiltration, less histological injury, a reduction in proinflammatory cytokine expression and decreased nitrosative stress. It was shown that a fluorescently labeled liposomal formulation of curcumin was endocytosed by antigen presenting cells and renal tubular cells [135]. In another study, under simulated I/R injury conditions water soluble distearoylphosphatidylethanolamine-polyethylene glycol nanoparticles carrying curcumin showed a cell protective effect on the proximal tubule epithelial cell line HK-2 cells by protecting against apoptosis protecting and reducing reactive oxygen species and malondialdehyde content [136]. There have been some studies on the combination of curcumin and stem cells in I/R injury that reported the restoring effect of one week intranasal administration of curcumin-loaded embryonic stem cell exosomes on the neurovascular unit induced by ischemia reperfusion injury in mice. Exosomes as the secretory vesicles of about 200 nm with the ability to cross blood-brain barrier and transfer cellular and biological components are suitable candidates for brain drug delivery [137, 138]. Curcumin loading was done with mixing and multiple rapid freeze-thawing. The presence of the fluorescently labeled curcumin-loaded embryonic stem cell exosomes was confirmed in different brain cellular compartments. This combination reduced post ischemic events manifested by neurological score, lesion volume, cerebral edema and inflammation. Other remarkable findings were the normalizing effect of this treatment on astrocytes and neuronal expression along with vascular inflammation and vascular junction breakdown reduction and endothelial vascular junction protein improvement [139]. Another group reported that oxidative stress induced injury in a rat model of myocardial ischemia reperfusion could be improved following the administration of adipose derived mesenchymal stem cells that had been primed with

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curcumin, reflected in improved heart function and neovascularization, decreased infarct size, and reduced myocardial apoptosis. This effect may have been contributed to by the antioxidant effect of curcumin protecting transplanted stem cells [140]. Soluble complexes of curcumin can be formed from cyclodextrins that are frequently used to solubilize and stabilize pharmaceutical agents as they have a lipophilic central cavity in their cyclic structure and hydrophilic outer surface. The combination of cyclodextrin and curcumin has been evaluated in a preclinical pig model of marginal kidney transplantation [141] and liver preservation [142]. In the kidney transplantation study, cyclodextrin-complexed curcumin used in the large white pig model showed enhanced function recovery, a reduction in histological injury and a twofold survival rate [141]. In the liver preservation study, it was concluded that flushing with cyclodextrin-complexed curcumin may be a promising approach to maintain the quality of the liver to be transplanted [142].

9. Conclusions

In this review, we have discussed the protective effects of curcumin ameliorating liver I/R injury. Many factors are involved in the process of liver I/R injury, including anaerobic metabolism, mitochondrial damage, oxidative stress, intracellular Ca^{2+} overload, cytokines and chemokines and neutrophils. Accumulating evidence suggests that curcumin improves liver I/R injury through different mechanisms such as anti-inflammation, reduction of oxidative stress and reduction of adhesion molecules. The main pathways involved in liver I/R injury are oxidative stress that is specifically targeted by curcumin by increasing antioxidant enzymes, decreasing ROS and increasing RNA scavenging. However, prospective studies are needed to further elucidate the protective effects of curcumin on liver I/R injury.

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Conflict of interest

The authors declare no conflict of interests.

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Table 1. Summary of studies reporting protective effects of curcumin against ischemia-reperfusion injury in the hepatic system.

Route of administration	Dose	Animal	Ischemia duration	Analysis time after hepatic injury	Time interval between curcumin administration and sample collection	Effect on hepatic IR injury	Ref
Oral gavage	200 mg/kg	Rat (250-300 g)	30 min	15 min	75 min	NO	[143]
Not defined	100 mg/kg	Male Sprague-Dawley rats (200–250 g)	1 hour	24 hours	7 hours and 30 min	YES	[10]
Injection through a branch of superior mesenteric	50 mg/kg	Sprague-Dawley male rats (220-300g)	45 min	30 min	3, 12, and 24 hours	YES	[90]
Not defined	15 mg/kg/d	Thirty Sprague-Dawley male rats (250-300 g)	30 min	15 minutes before ischemia and every 24 hours for the next 48 hours	48 hours and 45 min	YES	[144]
orally	25 mg/kg	24 Sprague‑Dawley rats (280‑330 g)	30 minutes	1 day before	25 hours and 50 min	YES	[30]

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Figure legends

Figure 1. Types of liver I/R injury. Two major types of liver I/R injury exist; the 'warm' I/R injury and the 'cold' I/R injury. The 'warm' I/R injury occurs during prolonged surgical liver resection and is initiated by hepatocellular damage whilst the 'cold' I/R injury occurs because of the ex vivo preservation of the donor organ that is kept cold during transplantation and is initiated by damage to hepatic sinusoidal endothelial cells and disruption of the microcirculation.

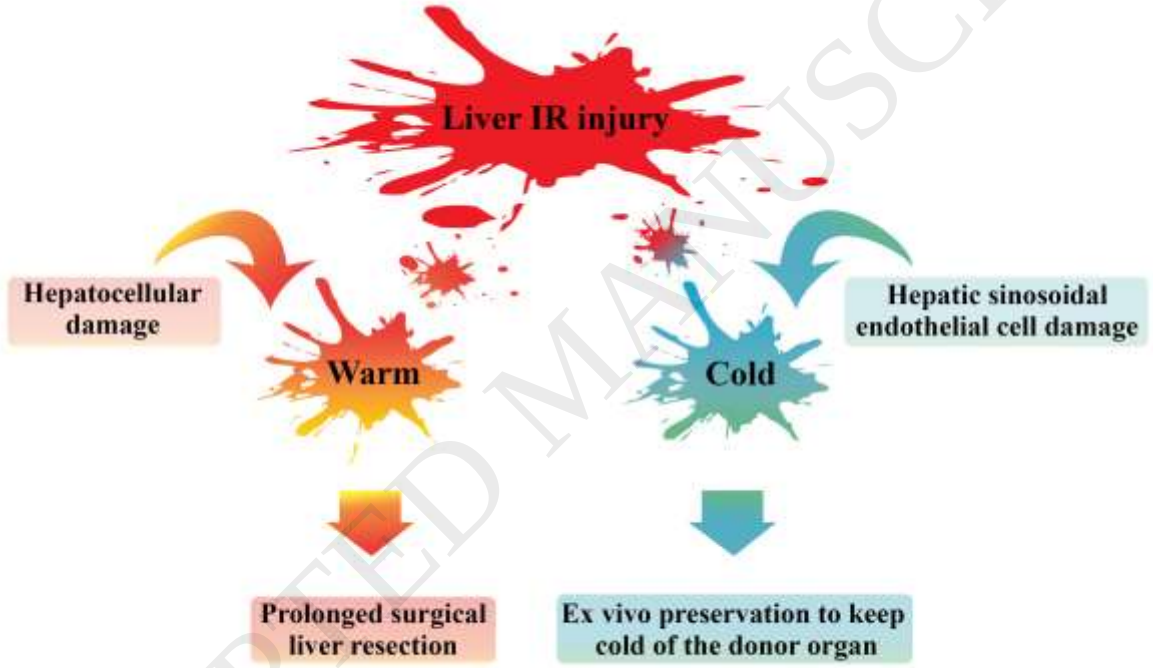

Figure 2. Biological effects of curcumin. Curcumin has various biological effects such as anti-oxidant, anti-inflammatory, antimicrobial, anti-tumour, nephroprotective, neuroprotective, antirheumatic, anti-cancer, anti-fibrosis activity, and attenuated liver injury.

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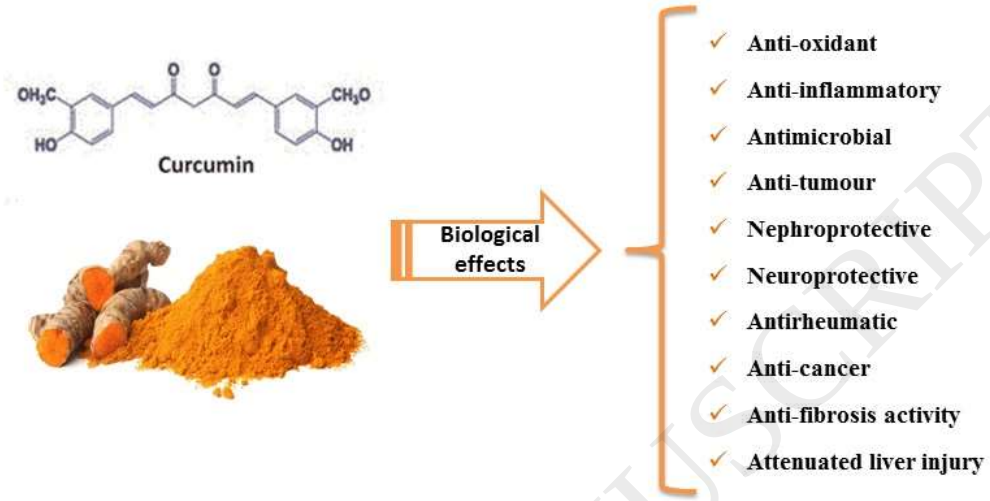

Figure 3. Schematic diagram showing the various mechanisms of liver ischemia reperfusion injury. The mechanisms of liver ischemia-reperfusion injury include oxidative stress, intracellular calcium overload, anaerobic metabolism, mitochondria dysfunction, liver Kupffer cells activation, vascular cell adhesion molecules overexpression, released pro-inflammatory cytokines and neutrophil influx.

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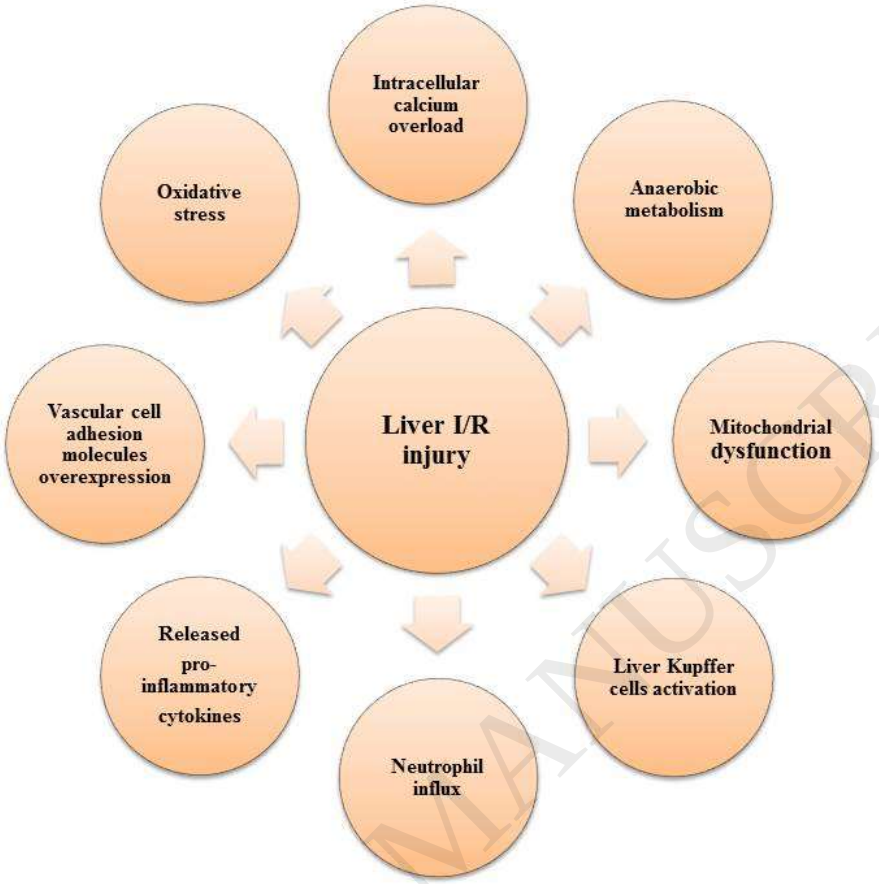

Figure 4. The effects of curcumin against liver I/R injury. Curcumin affects oxidative stress and inflammation through increasing antioxidant enzyme, ROS and RNA scavengers, increasing Hsp70, inhibition of NF-κB signaling and haemoxygenase inducer. Curcumin also affects immune cells through suppression of M1 Kupffer, promotion of M2 Kupffer, inhibition of neutrophil and infiltration. Curcumin alleviate liver I/R injury with these mechanisms.

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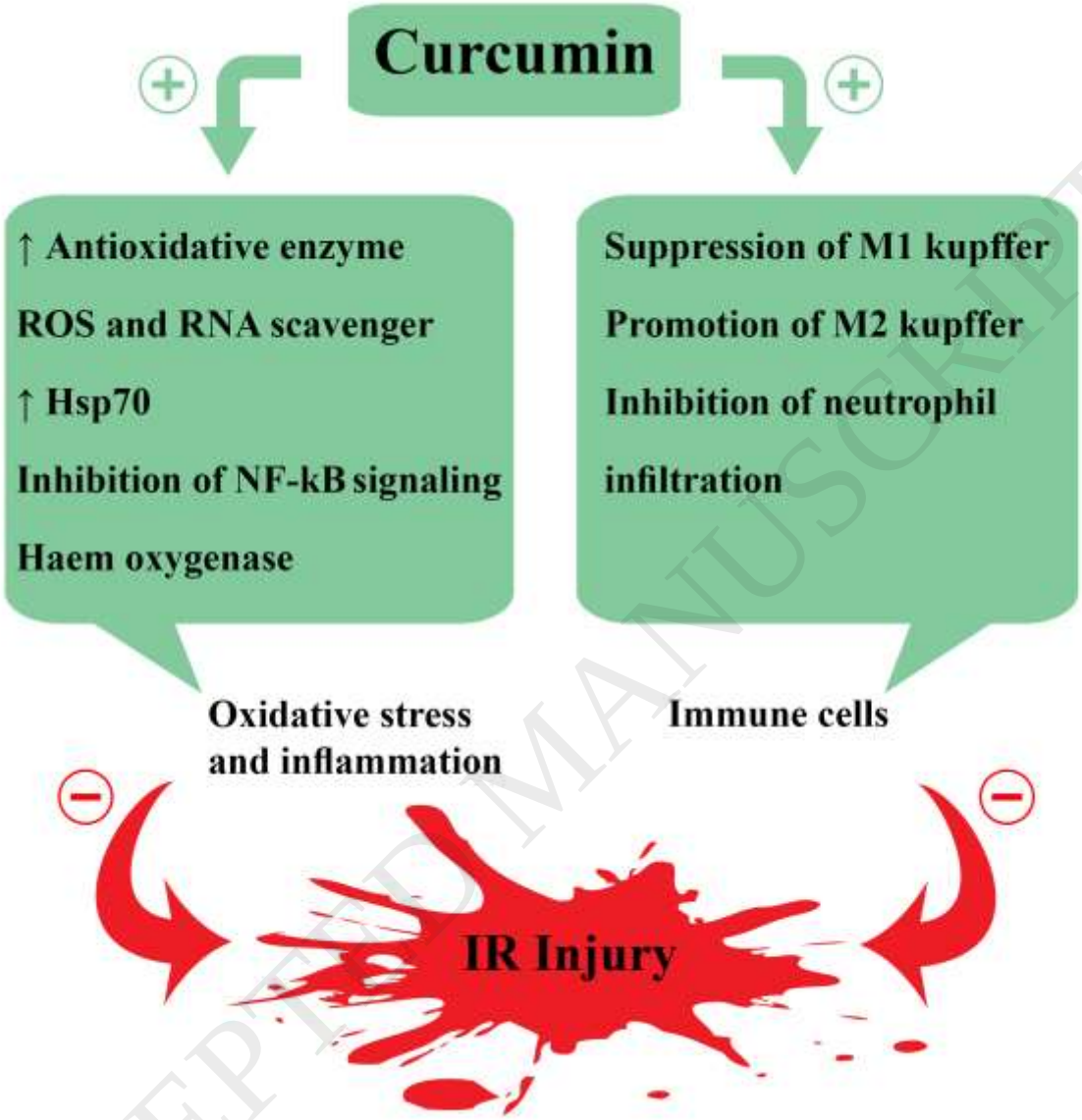

Figure 5. The effects of curcumin on important immune cells against liver I/R injury. One of the mechanisms of hepatic I/R injury alleviation by curcumin is suppression of M1 Kupffer cells, promotion of M2 polarization in these macrophages and inhibition of neutrophils by acting on oxidative stress, proinflammatory cytokines, chemokines and activated complement factors.

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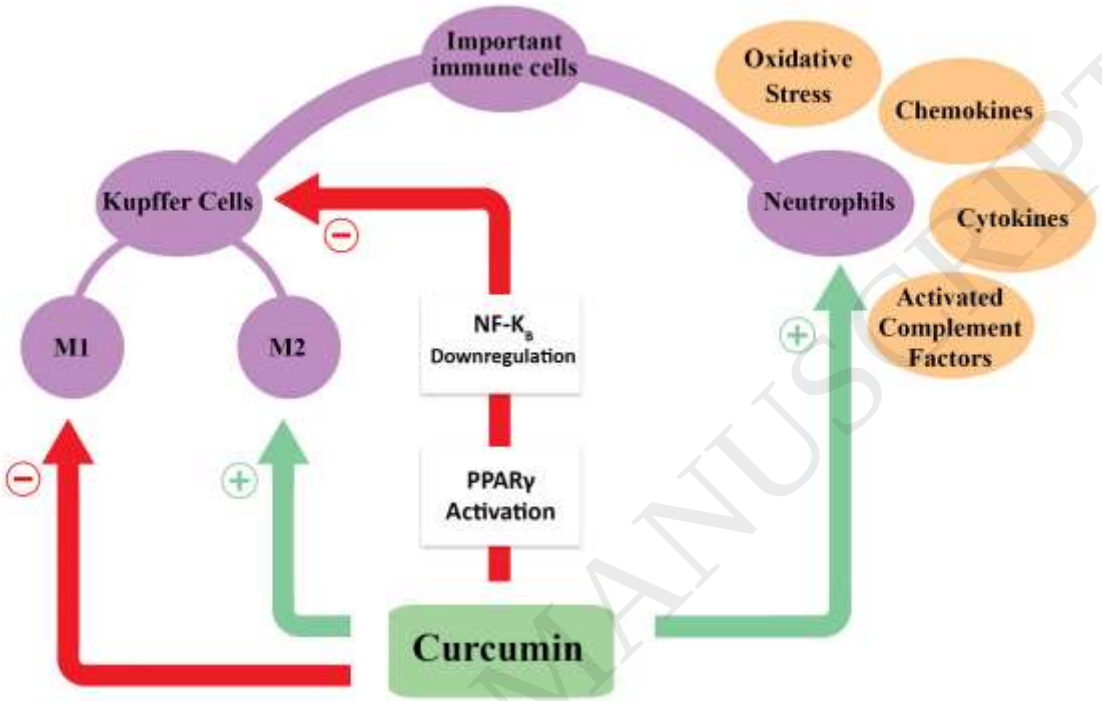

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