Thymoquinone and Curcumin

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D-galactose (D-gal) administration causes oxidative disorder and is widely utilized in aging animal models. Therefore, subcutaneously injected D-gal at 200 mg/kg BW dose to assess the potential preventive effect of thymoquinone (TQ) and curcumin (Cur) against the oxidative alterations induced by D-gal. Other than the control, vehicle, and D-gal groups, the TQ and Cur treated groups were orally supplemented at 20 mg/kg BW of each alone or combined. TQ and Cur effectively suppressed the oxidative alterations induced by D-gal in brain and heart tissues. The TQ and Cur combination significantly decreased the elevated necrosis in the brain and heart by D-gal. It significantly reduced brain caspase 3, calbindin, and calcium-binding adapter molecule 1 (IBA1), heart caspase 3, and BCL2. Expression of mRNA of the brain and heart TP53, p21, Bax, and CASP-3 were significantly downregulated in the TQ and Cur combination group along with upregulation of BCL2 in comparison with the D-gal group. Data suggested that the TQ and Cur combination is a promising approach in aging prevention.

Keywords: thymoquinone ; curcumin ; D-galactose ; oxidative stress ; anti-aging

1. Introduction

Aging is a deteriorative process that occurs mainly due to oxidative stress, leading to numerous oxidative stress-associated diseases because of the accumulation of reactive oxygen species (ROS) and reduced antioxidant capability [1][2]. A D-galactose (D-gal)-induced aging model is a commonly utilized model to investigate anti-aging drugs [3]. When D-gal accumulates in the body, it can react with the free amines of amino acids in proteins and peptides, forming advanced glycation end products (AGEs) [4]. Consequently, AGEs interact with specific receptors (RAGE) in many cell types and induce the activation of the downstream nuclear factor kappa-B (NF-κB), and other signaling pathways, resulting in ROS generation, which could accelerate the aging process [4][5]. The elevated ROS and reactive nitrogen species (RNS), including superoxide anion and nitric oxide, lead to cellular damages in protein, lipid, and DNA that are able to favor the development of different diseases, including tumors, neurodegenerative disorders, aging, and an inflammatory processes [6][7][8][9].

Natural compounds act as preventive antioxidant agents against different age-associated alterations [10]. Thymoquinone (TQ) is an active compound of *Nigella sativa* seeds with diverse biological activities such as antioxidant, antitoxic, anti-inflammatory, antidiabetic, and anticancer activities (Figure 1) [11][12][13][14][15]. TQ showed a positive elevation in liver glutathione levels and enhancement of total oxidant status of blood in a rat model with carbon tetrachloride-induced hepatotoxicity [16]. Also, TQ protects cardiac muscles against diabetic oxidative stress by upregulation of nuclear factor-erythroid-2-related factor 2 (Nrf2), which improved the antioxidant potential of the cardiac muscles and alleviated the inflammatory process [12]. Moreover, TQ alleviates the testicular damage in diabetic rats through its powerful antioxidant and hypoglycemic effects [11]. Additionally, TQ shows a regenerative potential for treating damaged peripheral nerves [17].

Curcumin (Cur) is a yellow pigment obtained from *Curcuma longa*, commonly used as a spice and food-coloring agent (Figure 1). It has preventive or putative therapeutic properties because of its anti-inflammatory, antioxidant, anti-aging, and anticancer potential [18][19][20][21][22][23][24].

2. Current Insights

Aging (senescence) is the loss of organ and tissue function gradually with time [25]. The losses of age-associated functions are because of the cumulation of oxidative damage macromolecules (proteins, DNA, and lipids) by ROS and RNS [26]. Senescent cells pile through aging and have been involved in enhancing various age-related diseases [27]. Senescence inducers led to upregulation of *p53*, which elevated the cyclin-dependent kinase inhibitor $p21^{(WAF1/CIP1)}$, mainly mediating G1 growth arrest [28].

The mechanism D-gal inducing aging is well recognized and based on generation of ROS and RNS that induce inflammation and apoptosis of different body cells [4][5]. In the current study, we assessed the aging and apoptotic markers due to D-gal administration and the protective role of TQ, Cur and their combination. D-gal significantly upregulated *p21* and *TP53*, leading to aging oxidative alterations in brain and heart tissues. D-gal-induced upregulation of *p21* and *p53* in mouse [29] and rat [30] models treated by D-gal. Moreover, western blot results revealed upregulation of the p53/p21 signaling pathway in mice's hippocampus [31].

TQ, Cur, and their combination significantly downregulated the increased expression of *p21* and *TP53* because of D-gal. Also, TQ is responsible for apoptosis induction in colorectal cancer by inhibiting the p53-dependent CHEK1 [32]. Some rationales suggest that curcumin's anti-aging function is due to its ability to postpone cellular senescence in cells building the vasculature [32].

An elevated ROS level accompanies humans' aging and higher animals in mitochondria, inducing apoptosis, lowering the functioning cells' number [33]. D-gal-stimulated brain aging exhibited changes in cognitive function and brain mitochondria [34]. Also, hypertrophy of the myocytes and myocytes' loss are characteristic foraging in the mammalian heart [35]. During heart failure and normal heart aging, necrosis and apoptosis mechanisms are involved in myocyte cell loss [36][37]. In the present study, D-gal induced necrosis and apoptosis of brain and heart tissues monitored by upregulation of apoptotic (*CASP-3* and *Bax* genes and caspase 3 protein) and downregulation of antiapoptotic (*Blc2* gene and protein) markers. In the same context, D-gal induced significant decreases in the Bax/Bcl-2 ratio and caspase-3 in mice's brains [38] and rats [39]. Also, D-gal markedly lowered the Bax/Bcl-2 ratio and caspase-3 in aging rats' cardiomyocytes [40]. In contrast, TQ and Cur attenuated the necrotic and apoptotic alterations of rats' brains and hearts, especially their combination. Similarly, Abulfadl et al. [41] stated that TQ prevented D-gal/AlCl3-induced cognitive decline by promoting synaptic plasticity and cholinergic function and suppressing neuronal apoptosis oxidative damage and neuroinflammation in rats. Also, curcumin minimized the alterations induced in Purkinje cells [42] and cleaved caspase-3 expression [43] in rats due to D-gal.

Calcium has a pivotal role in the neurodegeneration process and has an essential role as an intracellular signaling mediator [44]. Therefore, multiple injury pathways meet to stimulate an extra increase in intracellular calcium levels, inducing a series of caspases leading to the apoptosis onset. So, the calcium homeostasis maintenance within neurons is essential to their health, including many mechanisms [45]. Calbindin is a calcium-binding protein that protects neurons against damage caused by excessive Ca^{2+} elevation [46]. Thus, in the current investigation, D-gal induced reduced brain calbindin in rats leading to activation of caspase 3 that induced apoptosis of brain tissue, while TQ, Cur, and their combinations attenuated the calbindin reduction due to D-gal. There is no published article regarding the influence of D-gal or TQ on brain calbindin expression. At the same time, curcuminoid submicron particle consumption inverted spatial memory deficits and the loss of calbindin in the hippocampus of the Alzheimer's disease mouse model [47].

IBA1 is a cytoskeleton protein localized only in macrophages and microglia [48]. The IBA1 expression is upregulated in stimulated microglia after ischemia [49], peripheral nerve injury [50][51], and many brain diseases [52]. In the present investigation, we recognized a marked elevation in IBA1 expression in brain tissue. Similarly, D-gal significantly increased the neuroinflammatory marker, IBA1, in the mouse brain [53][54]. Conversely, TQ and Cur and their combinations significantly reduced the elevated IBA1 in response to D-gal.

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