JOURNAL OF FUNCTIONAL FOODS 5 (2013) 251–259

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jff

# Alleviation of plasma, erythrocyte and liver lipidemic-oxidative stress by thymoquinone and limonene in atherogenic suspension fed rats

*Shafeeque Ahmad**, *Zafarul H. Beg*

*Department of Biochemistry, Jawahar Lal Nehru Medical College, Aligarh Muslim University, Aligarh 202 002, India*

ARTICLE INFO

*Article history:*
Received 17 August 2012
Received in revised form
15 October 2012
Accepted 16 October 2012
Available online 11 November 2012

*Keywords:*
Thymoquinone
Limonene
Oxidative abnormalities
Lipid peroxidation
Atherogenic suspension
ATPases

ABSTRACT

In the present study, the antioxidant efficacies of thymoquinone (TQ) and limonene (LMN), two main constituents of *Nigella sativa* seeds, were investigated in relation to plasma, erythrocyte and liver oxidative abnormalities in hyperlipidemic Wistar albino rats. Pretreatment with 10 mg TQ or 200 mg LMN in atherogenic suspension fed rats, effectively reduced the plasma lipid peroxidation markers, conjugated diene, lipid hydroperoxide, malondialdehyde, and replenished the plasma antioxidant capacity by increasing its ferric reducing ability and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid quenching to near normal levels and modulating the levels of reduced glutathione, enzymatic antioxidants superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and erythrocyte membrane-linked ATPases to normalcy. These results demonstrate that radical scavenging/antiperoxidative efficacies of TQ were greater than LMN. Thus, these compounds, especially TQ, play an important and useful role in the preservation of plasma antioxidant status, cellular membrane structure and function of tissues, and may be used as chemopreventative food additives in the prooxidant state related disorders.


## 1. Introduction

Hypercholesterolemia has emerged as one of the most important risk factors for cardiovascular disease (CVD) (Steinberg, 2002). Increasing evidence shows a positive correlation between dietary saturated fat and plasma cholesterol levels, and feeding animals with a diet rich in cholesterol and saturated fat is associated with free radical production and lipid peroxidation, followed by oxidative stress and hypercholesterolemia (Bulur et al., 1995; Stehbens, 1986). Oxidative stress, which results from an imbalance between formation of free radicals and antioxidant defense systems, is one of the factors that links hypercholesterolemia with atherogenesis (Halliwell, 1996). Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by reactive oxygen species (ROS)/free radicals, which leads to accumulation of lipid peroxidation products such as malondialdehyde (MDA), hydrogen peroxide and also hydroxyl radicals, which in turn propagate lipid peroxidation, and cause serious damage to the membrane and changes in intracellular enzymes, resulting in loss of cell function and cell death (Kehrer, 1993; Pompella, Romani, Benedetti, & Comporti, 1991). Thus, free radical-mediated lipid peroxidation contributes to the etiology of a number of pathological conditions such as atherosclerosis, diabetes, inflammation and aging. Erythrocytes are constantly exposed to both extracellular and intracellular sources of ROS. They are extremely susceptible to oxidative damage induced by ROS, because they contain hemoglobin and polyunsaturated fatty acids (PUFAs), which are readily peroxidized (Yilmaz, Celik, Naziroglu, Cay, & Dilsiz, 1997). In

* *Corresponding author.* Tel.: +91 7669465144.
E-mail address: azamshafeeque1@gmail.com (S. Ahmad).
1756-4646/$ - see front matter 
http://dx.doi.org/10.1016/j.jff.2012.10.014

addition, hypercholesterolemia leads to increased cholesterol accumulation in the erythrocytes, which results in the production of excessive oxygen free radicals (Prasad & Kalra, 1989). Thus, exposure of erythrocytes to these substantially increased levels of ROS/free radicals is associated with a number of membrane changes including lipid peroxidation (Moore, Bamberg, Wilson, Jenkins, & Mankad, 1990; Sato, Kamo, Takahashi, & Suzuki, 1995), protein crosslinking (Moore et al., 1990), and sulfhydryl group oxidation (Soszynski & Bartosz, 1997), resulting in membrane damage and hemolysis (Sato et al., 1995). Membrane enzymes such as ATPases are also targets of free radical attack (Moore et al., 1990). Decrease in erythrocyte ATPase activities has been found to coincide with pathological changes of other clinical parameters in coronary heart disease (Zhou, Lin, & Guo, 1999). In order to protect the tissues from oxidative damage, organisms possess enzymatic and nonenzymatic antioxidant defense systems (Parthasarathy, Santanam, Ramachandran, & Meilhac, 2000). Protection against ROS and the breakdown products of peroxidized lipids and oxidized proteins is provided by enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), glutathione reductase (Gred), glutathione-S-transferase (GST) and nonenzymatic antioxidant, reduced glutathione (GSH). Therefore, a deficiency of these antioxidant systems produces a spontaneous ROS accumulation within cells.

In recent years, experiments have been performed on natural antioxidant compounds in order to prevent and/or reduce damage to biological tissues and are currently extensively investigated in the treatment of various diseases. Compounds, which possess antioxidant properties, have the potential to decrease oxidative stress and protect tissues from the detrimental effects of ROS/free radicals. Antioxidants have been detected in a number of food and agricultural products, including cereal grains, vegetables, fruits, and oil seeds (Burits & Bucar, 2000; Yu, Perret, Davy, Wilson, & Melby, 2002b; Yu et al., 2002a) Previous studies have reported the antioxidant activities of limonene (LMN) (Lado, Then, Varga, Szoke, & Szentmihalyi, 2004) and, principal active constituent of *Nigella sativa* seed, thymoquinone (TQ), against biologically hazardous ROS/free radicals (Houghton, Zarka, de la Heras, & Hoult, 1995). In the present study, the putative preventive effects of TQ and LMN were investigated on overall plasma antioxidant capacity, enzymatic and nonenzymatic antioxidant defense systems in erythrocytes and liver, lipid peroxidation in plasma, including ATPases in erythrocytes in the presence of lipidemic-oxidative stress, induced in rats by feeding an atherogenic suspension containing 5 mg cholesterol, 30 mg coconut oil and 2.5 mg cholic acid for 30 days. Cholic acid is the predominant bile acid, derived from cholesterol. This has detergent property which aids in digestion and absorption of lipids (Nelson & Cox, 2004).

## 2. Materials and methods

### 2.1. Chemicals

Thymoquinone, limonene, 1,1,3,3-tetramethoxypropane, ouabain and adenosine triphosphate were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA), while 2,4,6-tripyridyl-S-triazine, triphenyl phosphine, xylenol orange, nitroblue tetrazolium salt, phenazine methosulfate, NADP, NADPH, glutathione reduced and N-ethylmaleimide were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). 5,5′-dithiobis (2-nitrobenzoic acid) and glutathione oxidized were procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Phosphotungstic acid was purchased from Loba Chemie (Mumbai, India). 1-Chloro 2,4-dinitrobenzene (CDNB) was procured from Central Drug House, Pvt. Ltd. (Mumbai, India). Hemoglobin assay kit was purchased from Ranbaxy Diagnostics (New Delhi, India). Rat chow was procured from Ashirwad Industries (Chandigarh, India). All other chemicals and reagents used in this study were of analytical grade.

### 2.2. Animals and treatments

Wistar male albino rats, aged 3 months, weighing 180–210 g, from the central animal facility of J.N. Medical College, were taken. In this study, only male Wistar rats were used to avoid the secondary variability to sex differences. They are comparatively, less affected by hormonal changes. Due to the presence of less estrogen in male rats, blood lipids are not affected (Ganong, 2002). In addition, rats have similarities with humans in terms of physiology, pathology and metabolism. The protocol for this study was approved by the board of studies of the Biochemistry department and ethics committee of the Medical College. The rats were given pelleted rat chow and water *ad libitum*. In order to induce hyperlipidemia, animals in experimental groups received an atherogenic suspension for 30 days. This suspension consisted of (w/v) of 0.5% cholesterol, 3% coconut oil and 0.25% cholic acid, prepared by mixing in a Potter-Elvehjem homogenizer. One ml of this atherogenic suspension containing 5 mg cholesterol, 30 mg coconut oil and 2.5 mg cholic acid was administrated orally to each rat by using intragastric intubation in two divided doses (morning and evening) of 0.5 ml each. Five normolipidemic rats in control group (NLP-C) were given 0.5 ml of saline twice daily. For the treatment of diabetic neuropathy in male wistar rat, Kanter (2008) used 50 mg TQ/kg body weight/day (i.e. 10 mg/rat/day), orally. Four different doses of TQ were also given to male Wistar rat at 5, 20, 50 and 100 mg/kg body weight/day (i.e. 1, 4, 10, 20 mg/rat/day), orally (El-Abhar, Abdallah, & Saleh, 2003). These two high doses, 50 and 100 mg/kg body weight/day were quite effective. In the case of LMN dose selection, for the treatment of cancer in male wistar rat, van Lieshout, Posner, Woodard, and Peters (1998) used lab chow supplemented with 10 000 ppm *ad libitum* (i.e. 200 mg LMN/rat/day). On the above rationales, single and effective dose of 1% TQ and 20% LMN suspensions were prepared by dissolving in DMSO (12.5%) and then homogenizing with saline. Before 30 minutes administration of atherogenic suspension, rats in hyperlipidemic TQ (HLP-TQ) and hyperlipidemic LMN (HLP-LMN) groups received one ml of above saline suspension containing 10 mg of TQ or 200 mg of LMN by using intragastric intubation in two equal doses (morning and evening) of 0.5 ml each for 30 days, while rats in hyperlipidemic control (HLP-C) group, with no drug intervention, received 0.5 ml of saline containing 12.5% DMSO,