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Effect of Thymoquinone on MDA and SOD levels in Sterptozotocine Induced Diabetic Albino Rats.

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Abstract

Introduction - Generation of reactive oxygen species and lipid peroxidation are associated with tissue Ischemia or Reperfusion. Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. Their destruction can lead to cell death and to production of toxic and reactive aldehyde metabolites called free radicals. Among these free radicals, malondialdehyde (MDA) is the most important. To control the flux of reactive oxygen species (ROS) in physiological conditions, aerobic cells have developed their own defense system against free radical attacks, the antioxidant system, which includes both enzymatic and non-enzymatic components. This system consists of low molecular weight antioxidant molecules and various antioxidant enzymes, including, superoxide dismutase (SOD). Thymoquinone is major bioactive component of nigella sativa seed.

Materials and Methods -This work is conducted as part of Ph.D work under Department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Inistitution Animal Ethical committee are approved the work according to CPCSEA Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as normal control, one group served as Diabetic control and one groups served as Treatment group with Thymoquinone(4mg/kg BW).

Results -MDA(nmol/ml) level of Normal Control rats was 6.64±0.99, Diabetic rats was 12.70±1.54 and treated with Thymoquinone was 6.99±1.60. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with Thymoquinone rats was 4.15±1.14.

Conclusion -Compared with normal rats the level of MDA was increased in diabetic rats, when it is treated with Thymoquinone powder the levels of MDA reduced significantly. Compared with normal rats the level of SOD was decreased in diabetic rats, when it is treated with Thymoquinone the levels of SOD increased significantly.

Key Words – MDA, SOD, Thymoquinone, Antioxident.

INTRODUCTION

Thymoquinone, the major active constituent of NigellaSativa seeds, is a pharmacologically active quinone, which possesses several properties including analgesic and anti-inflammatory actions, protection against chemical induced carcinogenesis and the inhibition of eicosanoids generation [1,2]. In previous studies reported that thymoquinone prevents oxidative injury in hepatocytes induced by carbon tetrachloride or tert-butyl hydroperoxide in various in vitro and in vivo hepatotoxicity models, as well as acetic acid-induced colitis in rats. It has been suggested that thymoquinone may act as an antioxidant agent and prevent the membrane lipid peroxidation in hepatocytes[3,4,5].

Diabetes mellitus represents one of the greatest threats to modern global health and affects millions of people worldwide. DM is a chronic and progressive metabolic disorder characterized by hyperglycemia resulting from deficiency in insulin secretion, insulin action, or both[6]. The increased extra- and intracellular glucose concentrations result in oxidative stress, which seems to be due mainly to increased production of reactive oxygen species (ROS) and free radicals with a sharp reduction in antioxidant body defences[7]. Free radicals are continuously produced during normal physiologic processes and attack macromolecules including proteins, lipids, and DNA, so causing tissue injury. It has been widely accepted that oxidative stress plays a key role in the onset and development of diabetes complications, notably nephropathy[8]. Several mechanisms seem to be involved in the generation of oxidative stress in experimental animals and patients. These mechanisms include glucose autooxidation, peroxidation or glycation of proteins, lipids, and DNA. Oxidative stress can arise from a number of different sources, whether disease state or lifestyle, including episodes of ketosis, sleep restriction, and excessive nutrient intake[9].

In the past two decades, it has become increasingly clear that oxidative stress plays a major role in the pathogenesis of a number of human diseases such as atherosclerosis, chronic renal failure, ischemia/reperfusion injury, neurodegenerative diseases, hypertension, cancer and

diabetes mellitus[10]. Although the pathophysiology of diabetic complications is multifactorial, animal and human studies suggest a role for oxidative stress via an increased formation of reactive oxygen species [11,12]. The primary antioxidant enzyme system includes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Oxidative stress is a constant feature of uncontrolled diabetes in humans and animals[13]. Nonenzymatic antioxidants such as vitamin C and E are decreased in diabetes, suggesting that oxidative stress in diabetes is, at least in part, due to impaired antioxidant system. Additionally, reports from various laboratories on the activities of SOD, CAT and GPX have been controversial[14,15]. Oxidative stress has been shown to be involved in the pathogenesis of many different forms of genetic and acquired hypertension[16,17]. Poorly controlled longstanding diabetes frequently results in nephropathy and cardiovascular complications[18,19]. plored whether NS treatment protects against pancreatic -cell damage in STZ-induced diabetic rats.The antioxidant status of a cell determines its suscep-tibility to oxidative damage, and is usually altered in response to oxidative stress (Halliwell and Gutteridge,1999). Accordingly, there has been increasing interest regarding the role and use of natural antioxidants as a means of preventing oxidative damage in diabetes due to high oxidative stress (Pritchard et al., 1986). The seed of Nigella sativa L. (NS), an annual Ranunculaceae herbaceous plant, has been used for centuries in the Middle East, northern Africa, the Far East, and Asia as a traditional treatment for asthma. NS contains Ć30 w/w of a fixed oil, and 0.40 – 0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4 –24% thymoquinone and 46% monoterpenes, such as p-cymene and a-pinene (El-Tahir et al., 1993). Recently, clinical and experimental studies have demonstrated many therapeutic effects of NS extracts, including immunomodulative (El-Kadi and Kandil, 1987), antiinflammatory (Houghton et al., 1995), antitumour (El-Daly, 1998), antidiabetic (Al-Hader et al., 1993; El-Shabrawy and Nada, 1996; Kanter et al., 2003a), and antiulcerogenic (El-Dakhakhny et al., 2002) effects.Antioxidants (e.g., vitamins C and E, enzyme superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx)) have been shown to protect cells against lipid peroxidation, the initial step in many pathological processes (Williams, 1984; Bray and Bettger, 1990). Re duced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors (Grankvist et al., 1981; Kanter et al., 2003b).

The present study was undertaken to determine whether the pancreas is subjected to oxidative damage during diabetes, and to examine the accompanying changes in antioxidant status in order to elucidate its role in the pathogenesis of this disease. In addition, we explored whether NS treatment protects against pancreatic-cell damage in STZ-induced diabetic rats.

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MATERIALS AND METHODS

Study design - This work is conducted as part of Ph.D work under Department of Anatomy, Shri BM patil Medical College, BLDE University, Bijapur. University ethical committee and Inistitution Animal Ethical committee are approved the work according to CPCSEA Rules. 18 rats were selected for this study and divided in to 3 groups each contains 6 rats, one group served as negative control, one group served as Diabetic control and one groups served as Treatment group with Thymoquinone(4mg/kg BW), at the end of 45th day blood

was collated and measured MDA and SOD by manual method.

Thymoquinone – Thymoquinone purchaged from Sigma-Aldrich, Bangalore and administrated to rats through intraperitoneal injections(4mg/body Kg weight).

Streptozotocine – Induced diabetis -The rats were given Streptozotocine intraperitoneal injection 50mg/BW, Streptozotocine dissolved in icecold citrate buffer(PH 4.5). The diabetes was confirmed by measuring glucose by Code free Glucometer, the glucose level above 250mg/dl considered as diabetes, glucose levels were checked at regular periodical timings.

RESULTS

MDA(nmol/ml) level of Normal Control rats was 6.64±0.99, Diabetic rats was 12.70±1.54 and treated with Thymoquinone rats was 6.99±1.60. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with Thymoquinone rats was 4.15±1.14.

One way results of MDA(nmol/ml) and SOD(U/ml)

Parameter	Group 1 Normal Rats – Control	Group 2 Diabetic Rats – Control	Group 3 Diabetic Rats – Thymoquinone(4 mg/KgBW)	F - Value	P
MDA(nmol/ml)	6.64± 0.99[a]	12.70± 1.54[b]	6.99±1.60[c]	24.276	<0.0001
SOD(U/ml)	4.91± 0.72[a]	1.57±0.27[b]	4.15±1.14[c]	19.52	<0.0001

The difference between groups P<0.05 considered as significant.

DISCUSSION

MDA(nmol/ml) level of Normal Control rats was 6.64±0.99, Diabetic rats was 12.70±1.54 and treated with thymoquinone(4mg/KgBw) rats was 6.99±1.60. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with thymoquinone(4mg/KgBw) rats was 4.15±1.14. Our results are in agreement with studies of Tuncel N et al[22], Kanter M et al[21], A.A. Sayed[23], Bassem Y et al[24], Dalia A. Hafez[25], Desai S D et al[26]. ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanism. In pathological conditions, ROS are over produced and result in lipid peroxidation and oxidative damage. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules[22].

Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products, MDA and 4-hydroxynonenal[27]. In present study the serum MDA levels significantly increased in the diabetic group with a reduction in the antioxidant enzyme activities of SOD. Nigella Sativa treatment decreased the elevated MDA and also increased the reduced SOD antioxidant enzyme activities. Our results are in agreement with studies of Wolf [28], El-Missiry and El-Gindy[29], and Mahmood et al. [30] these studies were reported an increase in lipid peroxides and a decrease in antioxidant enzymes in Diabetes. Schettler et al. suggested that the reduced antioxidant production was due to increased oxygen metabolities causing a decrease in the activity of the antioxidant defence system[31]. Kennedy and Baynes reported that decreased antioxidant enzyme activity in Diabetes is due to non-enzymatic glycosylation of the enzymes[32]. The present study is confirmed that thymoquinone may have antioxidant properties that will be useful for therapeutic purposes. The results of the present study indicate that the preventive effects of thymoquinone may be due to inhibition of lipid peroxidation as a result of its antioxidant nature.

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