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Effect of Nigella Sativa Seed Powder on MDA and SOD levels in Sterptozotocine Induced Diabetis Albino Rats.

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Abstract

Introduction –
MDA is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. Superoxide is produced as a by-product of oxygen metabolism and causes many types of cell damage. SOD is an important antioxidant defence in nearly all living cells exposed to oxygen. The seeds of Nigella Sativa known as black cumin seed, have long been used in the Middle East as a traditional medicine for a variety of complaints, headache, cough, flatulence, as a choleretic, antispasmodic and uricosuric. In recent years, the seeds have been subjected to a range of pharmacological investigations. Nigella Sativa is presently used in tradational medicine and for culinary preparations in many countries. The present study is conducted to estimate the effect of Nigella Sativa seed powder on Serum MDA and SOD levels in Streptozotocine Induced Diabetic Rats.

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 Nigella Sativa seed powder(300mg/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 nigella sativa rats was 7.39±1.05. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with nigella sativa rats was 3.70±0.73.

Conclusion –
Compared with normal rats the level of MDA was increased in diabetic rats, when it is treated with Nigella Sativa Seed 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 Nigella Sativa Seed powder the levels of SOD increased significantly.

Key Words – MDA, SOD, Nigella Sativa, Antioxident.

INTRODUCTION

Diabetes mellitus is associated with endothelial dysfunction and oxidative stress[1,2]. Chronic exposure to elevated glucose and fatty acid concentrations can cause damage in different types of cells by a variety of mechanisms collectively known as glucolipotoxicity, and oxidative stress may be a common link[3]. The oxidative stress in DM is greatly increased due to prolonged exposure to glycaemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of oxidative stress[4]. Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of DM[5]. Malondialdehyde (MDA) is a major player in lowdensity lipoprotein (LDL) modification and is a product of the peroxidation of arachidonic, eicosapentaenoic and docosahexaenoic acids[6]. Oxidised-LDL results from the interactions

between aldehydes such as MDA and lysine residues in apoB-100 of LDL[7]. The pathologic effects of Oxidised-LDL include the induction of atherosclerosis[8] atherothrombosis[9] and plaque erosion[10].

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[11]. 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 [12,13]. 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[14]. 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[15,16]. Oxidative stress has been shown to be involved in the pathogenesis of many different forms of genetic and acquired hypertension[17,18]. Poorly controlled longstanding diabetes frequently results in nephropathy and cardiovascular complications[19].

The Nigella sativa seed, known as 'Black Seed' is frequently used in many parts of the world, particularly in the Middle-East and Far-East countires, for the prevention and treatment of a large number of diseases[20]. The Nigella sativa seed and its active constituents possess many pharmacological properties, including antioxidant, antiinflammatory, analgesic, antipyretic, antiasthmatic, antihypertensive, antimicrobial and antineoplastic[21,22]. Most of the biological effects of N. sativa seed are shown to be due to its major active principle, thymoquinone[23].

plored whether NS treatment protects against pancreatic ␤-cell damage in STZ-induced diabetic rats.

The antioxidant status of a cell determines its susceptibility to oxidative damage, and is usually altered in response to oxidative stress (Halliwell and Gutteridge,1999). Accordingly, there has been increasing interest re-garding 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). Reduced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors (Grankvist et al., 1981; Kanter etal., 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.

The antioxidant status of a cell determines its susceptibility 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 herba ceous 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.

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The antioxidant status of a cell determines its susceptibility to oxidative damage, and is usually altered in response to oxidative stress. 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. Nigella Sativa contains _30 w/w of a

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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[25]. Reduced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors[26]. The present study was undertaken to determine the MDA and SOD levels in Streptozotocine induced diabetic albino rats.

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 Nigella Sativa seed powder(300mg/kg BW), at the end of 45th day blood was collated and measured MDA and SOD by manual method.

Plant material – Nigella sativa seeds were grinded in to fine powder[27] with piston and mortal with help of Bapuji pharmacy college, Davangere. Nigella sativa powder administrated orally according to study of M. Murugesan[28].

Streptozotocine – Induced diabetis -The rats were given Streptozotocine intraperitoneal injection 50mg/BW, Streptozotocine dissolved in icecold citrate buffer(PH 4.5)[29]. 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 periods.

RESULTS

MDA(nmol/ml) level of Normal Control rats was 6.64±0.99, Diabetic rats was 12.70±1.54 and treated with nigella sativa rats was 7.39±1.05. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with nigella sativa rats was 3.70±0.73.

DISCUSSION

MDA(nmol/ml) level of Normal Control rats was 6.64±0.99, Diabetic rats was 12.70±1.54 and treated with nigella sativa rats was 7.39±1.05. SOD(U/ml) level of Normal Control rats was 4.91±0.72, Diabetic rats was 1.57±0.27 and treated with nigella sativa rats was 3.70±0.73. Kanter et al studied the effect of black seed on lipid peroxidation and antioxidant defense system and found that treatment with the volatile oil of Nigella sativa decreased blood MDA levels and increased the antioxidant defense system activity in carbon tetrachloride treated rats[30]. The another study of Kanter showed that NSO treatment reduced the spinal cord tissue MDA and prevented from inhibition of SOD, GPX, and catalase (CAT) enzyme activities, following the experimental spinal cord injury in rats[31].

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[32]. 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 [33], El-Missiry and El-Gindy[34], and Mahmood et al. [35] 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[36]. Kennedy and Baynes reported that decreased antioxidant enzyme activity in Diabetes is due to non-enzymatic glycosylation of the enzymes[37]. The present study is confirmed that Nigella Sativa may have antioxidant properties that will be useful for therapeutic purposes. The results of the present study indicate that the preventive effects of Nigella Sativa may be due to inhibition of lipid peroxidation as a result of its antioxidant nature.

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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 – Nigella sativa seed powder	F - Value	P
MDA (nmol/ml)	6.64±0.99^{a}	12.70±1.54^{b}	7.39±1.05^{c}	24.276	<0.0001
SOD(U/ml)	4.91±0.72^{a}	1.57±0.27^{b}	3.70±0.73^{c}	19.52	<0.0001

The difference between groups P<0.05 considered as significant.

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