Antioxidant Effect of Nigella Sativa Seed Powder and Thymoquinone in Normal and Sterptozotocine Induced Diabetic Albino Rats

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ABSTRACT

Introduction: Malondialdehyde (MDA) cause toxic stress in cells and form covalent protein and its used as a biomarker to measure the level of oxidative stress. There is opposite mechanism which control the flux of reactive oxygen species (ROS) called as antioxidant system, which includes both enzymatic andnon-enzymatic components. The important enzyme of this system is superoxide dismutase (SOD). The black cumin or Nigella Sativaseeds are used in traditional medicine of different countries. Thymoquinone is major bioactive component of nigellasativa seed and it is the cause of therapeutic property of Nigella sativa seed. The present study is conducted to see the effect of Nigella Sativa seed powder and Thymoquinone on Serum MDA and SOD levels in normal and Streptozotocine Induced Diabetic Rats.

Materials and Methods: 36 rats were selected for this study and divided in to 6 groups each contains 6 rats, one group served as normal control, one group served as normal rats received the nigella sativa seed powder(300mg/ Kg BW), one groups served as normal rats received the Thymoquinone(4mg/kg BW), one group served as Streptozotocine(50mg/kg BW) induced diabetic control rats, one group served as diabetic rats received the nigella sativa seed powder(300mg/Kg BW) and one groups served as diabetic rats received the Thymoquinone(4mg/kg BW).

Results: There is no change in MDA and SOD levels in normal rats treated with nigella sativa seed powder and thymoqinone. The levels of MDA are increased significantly in diabetic rats compared with normal rats, when the diabetic groups treated with nigella sativa seed powder and thymoqinone the MDA levels are decreased significantly (p<0.05). The levels of SOD are decreased significantly in diabetic rats compared with normal rats, when the diabetic groups treated with nigella sativa seed powder and thymoqinone the SOD levels are increased significantly.

Conclusion: The results of these study concluding that nigella sativa seed powder and thymoquinone has having antioxidant effect in diabetic rats which lowering the MDA levels and increasing the SOD levels in streptozotocine induced diabetic rats.

KEY WORDS: MDA, SOD, Nigella Sativa seed, Antioxidant. Thymoquinone.

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INTRODUCTION

The one the most important and frequently using seed in traditional medicine many parts of the world, particularly in the Middle-East and Far-East countries, for the prevention and treatment of a large number of diseases is nigella sativa seed also popularly known as Black Seed. The active components of nigella sativa seeds having the great pharmacological properties, including, antidiabetic, antioxidant, anti-inflammatory, analgesic, antipyretic, antiasthmatic, antihypertensive, antimicrobial and antineo-plastic [1,2]. The holistic medicinal property of nigella sativa seed is due to its major bioactive component thymoquinone [3]. Thymoquinone got its medicinal property due to its nature of pharmacologically active guinone, which possesses several properties including analgesic and anti-inflammatory actions, protection against chemical induced carcinogenesis and the inhibition of eicosanoids generation [4, 5]. Oxidative stress is leads to oxidative damage of cell which can measure by antioxidant status.

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 fixed oil, and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% thymoguinone and 46% monoterpenes, such as p-cymene and a-pinene [6]. 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 [7]. Reduced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors [8]. The present study was undertaken to determine the MDA and SOD levels in Streptozotocine induced diabetic albino rats.

The major active constituent of NigellaSativa seed is Thymoquinone, it is a pharmacologically active quinone, it has possesses several properties including analgesic and antiinflammatory actions, protection against chemical induced carcinogenesis and the inhibi-tion of eicosanoids generation [9,10]. 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. In previous studies it has been suggested that thymoquinone may act as an antioxidant agent and prevent the membrane lipid peroxidation in hepatocytes[11-13].

Type 2 diabetes is the most prevalent and serious metabolic disease all over the world, and its hallmarks are pancreatic beta cell dysfunction and insulin resistance. Under diabetic conditions, chronic hyperglycaemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate beta cell function and increase insulin resistance which leads to the aggravation of type 2 diabetes. In addition, chronic hyperglycaemia and ROS are also involved in the development of atherosclerosis which is often observed under diabetic conditions. Taken together, it is likely that ROS play an important role in the development of type 2 diabetes and atherosclerosis. It has been shown that ROS are produced in various tissues under diabetic conditions [14,15]. There are several sources of ROS in cells such as the nonenzymatic glycosylation reaction [16], the electron transport chain in mitochondria [17], and membrane-bound NADPH oxidase [18,19]. In diabetic animals, glycation reaction is observed in various tissues and organs, and various kinds of glycated proteins such as glycosylated haemoglobin, albumin, and lens crystalline are produced in a non-enzymatically manner through the glycation reaction. The reaction produces Schiff base, Amadori product, and finally advanced glycosylation end products (AGEs). During the process, ROS are also produced. The electron transport chain in mitochondria is also an important pathway to produce ROS. Under diabetic conditions, electron transport chain is activated, which leads to production of larger amounts of ROS. It has been shown that membrane-bound NADPH oxidase is also an important source of ROS.

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 [20]. Free radicals are continuously produced during normal physiolo-gic 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 [21]. Several mechanisms seem to be involved in the generation of oxidative stress in experimental animals and patients. These mechanisms include glucose autoxidation, 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 [22]. 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[23-27]. The present study is focused to find out nigella sativa seed powder and thymoquinone effect on MDA and SOD in normal and streptozotocine induced diabetic rats. The aim of present study is to observe the antioxidant property of Nigella Sativa seed and its major bioactive component Thymoguinone in normal and streptozotocine induced diabetic 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 (BLDEU/Dept of pharmacology 602/13). The 36 rats were selected for this study and divided in to 6 groups each contains 6 rats, 3 groups are

normal rats out of that one group served as normal control rats, one group was treated with nigella sativa seed powder(300mg/Kg BW weight), one group was treated with thymoquinone (4mg/ kg body weight). Other three groups were induced diabetic by single rapid intraperitoneal injection of streptozotocine (50mg/ kg body weight) streptozotocine out of that one group served as diabetic control, one group as diabetic treated with nigella sativa and one group served as diabetic rats treated with thymoquinone, at the end of 45th day blood was collated and measured MDA (Nadiger et al method) [28] and SOD (Marklund and Marklund) [29].

Plant material: Nigella sativa seeds were purchased from Safa honey & Co, Bangalore and 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 [30].

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 every day morning.

RESULTS

MDA (nmol/ml) level of Normal Control rats was 6.64 ± 0.99 , normal rat treated with Thymoquinone rats was 6.45 ± 0.73 , diabetic rat treated with nigella sativa rats was 6.51 ± 1.11 . Diabetic control rats was 12.70 ± 1.54 , diabetic rat treated with Thymoquinone rats was 6.98 ± 1.60 , diabetic rat treated with nigella sativa rats was 7.39 ± 1.05 . SOD(U/ml) level of Normal

Table 1: One Way ANOVA Results of MDA, SOD.

	Parameter	Groups								
		Normal Rats - Control	Normal Rats - Nigella Sativa Seed Powder	Normal Rats - Thymoquinone	Diabetic Rats - Control	Diabetic Rats - Nigella sativa seed powder	Diabetic - Thymoquinone	F	Ρ	
	MDA (nmol/ml)	6.63 <u>+</u> 0.99	6.51 <u>+</u> 1.11	6.45 <u>+</u> 0.73	12.70 <u>+</u> 1.53	7.39 <u>+</u> 1.04	6.98 <u>+</u> 1.60	24.276	0	
	SOD (U/ml)	4.91 <u>+</u> 0.71	5.01 <u>+</u> 0.71	5.11 <u>+</u> 0.64	1.57 <u>+</u> 0.27	3.70 <u>+</u> 0.72	4.14 <u>+</u> 1.14	19.521	0	

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Control rats was 4.91 ± 0.71 , normal rat treated with Thymoquinone rats was 5.11 ± 0.64 , diabetic rat treated with nigella sativa rats was 5.01 ± 0.71 . Diabetic control rats was 1.57 ± 0.27 , diabetic rat treated with Thymoquinone rats was 4.14 ± 1.14 , diabetic rat treated with nigella sativa rats was 3.70 ± 0.72 (Table 1).

Graph 1: Vitamin MDA (nmol/ml).



NC- Normal Control Rats, NNS- Normal rats treated with NIgella Sativa, NTQ- Normal rats Treated with Thymoquinone, DC- Diabetic Control Rats, DNS- Diabetic rats treated with NIgella Sativa, DTQ-Diabetic rats Treated with Thymoquinone.



NC- Normal Control Rats, NNS- Normal rats treated with NIgella Sativa, NTQ- Normal rats Treated with Thymoquinone, DC- Diabetic Control Rats, DNS- Diabetic rats treated with NIgella Sativa, DTQ-Diabetic rats Treated with Thymoquinone.

DISCUSSION

In present study MDA (nmol/ml) level of Normal Control rats was 6.64 ± 0.99 , normal rat treated with Thymoquinone rats was 6.45 ± 0.73 , diabetic rat treated with nigella sativa rats was 6.51 ± 1.11 . Diabetic control rats were 12.70 ± 1.54 , diabetic rat treated with Thymoquinone rats was 6.98 ± 1.60 , diabetic rat treated with nigella sativa rats was 7.39 ± 1.05 . Abdelmeguid NE et al study also shown similar findings that MDA levels are increased in diabetic rats and after treated with Nigella sativa extract MDA levels are decreased [31]. In study of Edibe Sariciceka, et al. observed decreased levels of MDA when treated with nigella sativa and thymoquinone [32]. In study of Yasin TULUCE also observed same results [33]. 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 defence system activity in carbon tetrachloride treated rats [34].

In present study SOD(U/ml) level of Normal Control rats was 4.91+0.71, normal rat treated with Thymoguinone rats was 5.11+0.64, diabetic rat treated with nigella sativa rats was 5.01+0.71.Diabetic control rats was 1.57+0.27, diabetic rat treated with Thymoguinone rats was 4.14+1.14, diabetic rat treated with nigella sativa rats was 3.70+0.72. Hanene Jrah Harzallah et al study shows that after treated with thymoguinone and nigella sativa SOD levels [35]. In study of Nabila E Abdelmeguid et al found similar findings [36]. Our results are in agreement with studies of Tuncel N et al [37], Kanter M et al [38], A.A. Sayed [39], Bassem Y et al [40], Dalia A. Hafez [41]. 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 [36]. The present study results are confirmed that Nigella sativa seed powder and thymoguinone 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 seed and thymoguinone may be due to inhibition of lipid peroxidation as a result of its antioxidant nature.

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