Antioxidant Effect of *Spirulina* in Long Evans Rat

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Abstract: *Spirulina*, a naturally occurring micro algae, was suspended in water and administrated orally to streptozotocin induced diabetic and nondiabetic rats at a dose of 400 mg per kg body weight. The animal of the control group was administrated extract of *Spinacea oleracea* (locally known as Palong Shak). Thirty days after the oral administration all the rats were sacrificed for the determination of super oxide dismutase. *Spirulina* increased the level of oxidative stress responsive enzyme Super oxide dismutase (SOD) in erythrocyte by 17.5% ($p<0.0005$) in diabetic rats suggesting that the herb may have antioxidant properties.

Key words: Diabetes Mellitus, Spirulina, Rat

INTRODUCTION

Cell damage is induced by reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them. Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. The main source of ROS *in vivo* is aerobic respiration, although ROS are also produced by peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes.[1,2]

Under normal conditions, ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, or glutathione (GSH) peroxidase. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, cancer, and aging.

Superoxide Dismutase (SOD) catalyzes the reduction of superoxide anions to hydrogen peroxide. Km for O_{2^-} for bovine SOD = 0.35mM

Covalent conjugation of superoxide dismutase with polyethylene glycol (PEG) has been found to increase the circulatory half-life and provides prolonged protection from partially reduced oxygen species.[3]

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MATERIALS AND METHODS

Experimental design. Twenty male Long Evans rats, Purchased from BIRDEM were used to carry out

the investigation. The rats were divided into four groups of five rates in each group. Group I was normal and group II was diabetic, group III was diabetic control and group IV was used to find out effect.

Source of *Spirulina*. Applied Botany Department, BCSIR, Dhaka, Bangladesh.

Induction of diabetes. Rats were fasted for 18 hours and then streptozotocin dissolved in physiological saline adjusted to pH 4.3 with 0.05 citric acid, injected intravenously at a dose of 60 mg per kg body weight. After 48 hours the rats were sacrificed, Blood was saved for SOD assay.

Collection of blood from tail vein. All rats were fasted fot 18 hours before blood was collected from tail vein, which was warmed for 30 seconds at 40°C in a water bath prior to cutting by a surgical scissors.

Administration of *Spirulina* suspension. Spirulina was suspended in water and this suspension was fed orally by a tube attached to a syringe to the experimental rats at a dose 400 mg per body weight.

Administration of *Spinacea oleracea* - suspension. Five gm of semisolid extract was suspended in water and fed orally to the experimental control rats at a dose of 400 mg per body weight.

Estimation of blood glucose. Blood glucose was estimated by the method of Lohr and Waller.[4] Here copper was reduced by glucose and was reacted with arsenomolybdate to form the color product which was estimated spectrophotometrically at 680 nm.

Estimation of super oxide dismutase activity in erythrocytes. Red cell super oxide dismutase activity was assayed by the method of Winterbourn, Hawkins,Christine, Maureen, Brain and Carrell.[4] This method depends on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by the superoxide radical, which was generated by the reaction of photoreduced riboflavin and oxygen.

RESULTS AND DISCUSSION

Superoxide dismutase activity has been measured in tissue of animals with chemically induced diabetes. Matkovics *et al.*[6] found that rata with STZ-induced diabetes had decreased total SOD activity in liver, kidney, spleen, heart, testis, pancreas, skeletal muscle and erythrocytes,[6] total SOD activity was not changed in brain or lung. Crouch *et al.*[7] found that rats with STZ-induced diabetes had decreased Cu-Zn SOD activity in erythrocytes and retina.[7] This study found no change in SOD activity in lung, liver, brain, aorta, kidney, whole eye and lens of rats with STZ-induced diabetes.[7] A third group of investigators found that Cu-Zn SOD activity was depressed in renal cortex and large and small bowel mucosa of STZ-induced diabetic rats.[8] Loven *et al.*[8] found that Mn SOD activity was unchanged in intestinal mucosa but was increased in the renal cortex of diabetic animals (8).Cu-Zn SOD activity was found to be decreased in renal cortex and jejunum of STZ- induced diabetic rats ad libitum.[9] Both oral glutathione and intramuscular insulin injection elevated the C-Zn SOD activity. The substrate for SOD, the superoxide radical, has been measured in the white cells of diabetics in a number of studied. Nath *et al.*[9] found that superoxide was significantly elevated in polymorphonuclear leucocytes (PMNs) from diabetic patients as compared to normal subjects.[9] This elevation was attributed to the reduction in the activities of the cytoplasmic and mitochondrial superoxide dismutase and the effect being more pronounced in the cytoplasmic fraction.

It is well known that glucose can react nonenzymatically with amino group on proteins in vivo and in vitro. Such a nonenzymatic glycation reaction occurs in various kids of proteins under physiological conditions, and modification of these proteins often has significant effects on their functions of physical properties.[8] It has been shown that the percentage of glycated Cu-Zn SOD was increased in insulin- dependent diabetic children. The percentage of glycated Cu-Zn SOD in the diabetic patients was 40.0 $\pm$ 8.2% significantly higher than

that in the control.[8] The specific of glycated Cu-Zn SOD in the diabetic patients was half than that in the control, again indicated that the glycated that the glycated from of Cu-Zn SOD was less active. It has been shown that Cu-Zn SOD was inactivated by glycation and that the principal glycated sites were Lys^{122} and Lys^{128}, which are located in the active site binding loop region. The computer image of the enzyme molecule indicated that Lys^{122} and Lys^{128} are located on the surface of the molecule and appear to be easily attacked by glucose. Spirulina increased the reduced glutathione level 24.99mg/100 ml hemolysis.[5] Reduced glutathione in addition to important function in secretion of xenobiotics is a potent antioxidant. Along with other antioxidant such as vitamin E and C, glutathione plays an important role in protecting islets cells of the pancreas from oxidative stress and damage.

Table 1. Effect of oral administration of Spirulina on Erythrocytes Superoxide dismutase levels of STZ-induced Diabetic Long Evans Rats.

Parameters assayed	Normal		Streptozotocin diabetic rats feed						Effect of *Spirulina* (%)	Significance
			None N=5		*S. oleracea Extract* N=5		*Spirulina* Suspension N=5			
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.		
Blood Glucose (mmol/L)	4.51	0.22	9.29	0.18	9.04	0.18	7.59	0.06	-18.29	P < 0.005
Erythrocytes Superoxide Dismutase levels (Unit/g of Hb)	4171.20	158.33	2854.20	136.60	2934.20	77.08	3449.90	159.00	+17.50	P < 0.0005

Insulin might directly induce the synthesis of SOD or active a proenzyme. An insulin- activated NADPH oxidase has been identified in adipocytes which generate hydrogen peroxide and superoxide radical. Induction of Mn-SOD in neonatal rat lung by superoxide radical has been reported previously.[10] Likewise, the absence of insulin may in some lead to the inhabitation on increased degradation of SOD.

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