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Antidiabetic Activity of *Basella rubra* and its Relationship with the Antioxidant Property

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Research Article

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ABSTRACT

Oxidative stress induced by streptozotocin (STZ) has been shown to damage pancreatic beta cell and produce hyperglycemia in rats. In the present study an attempt was made to examine the action of *Basella rubra* against experimental diabetes as well as the antioxidant potential of the leaf extract. Aqueous extract of *Basella rubra* (400mg/kg body weight for 30 days) was found to significantly reduce the blood sugar level. The oxidative stress produced by streptozotocin was found to be significantly lowered when compared to control rats. This was evident from a significant decrease in blood sugar level and increased level of liver enzymatic (Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx)) and non enzymatic antioxidants (vitamin C, E and reduced glutathione). These results indicate that *Basella rubra* extract effectively reduced the oxidative stress induced by streptozotocin and potential reduction in blood sugar level.

Keywords: Basella rubra, diabetes mellitus, antioxidant, oxidants, hyperglycemia;

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ABBREVIATIONS

SOD - Super oxide dismutase; CAT – Catalase; GPx - Glutathione peroxidase; B. rubra - Basella rubra; GSH - Glutathione; STZ - Streptozotocin

1. INTRODUCTION

Oxidative stress is produced during normal metabolic process in the body as well as a variety of environmental factors and chemical substances. Oxidative stress has been shown to have a significant effect in the causation of diabetes mellitus as well as diabetes related completions in human beings (Wilson, 1998). Oxidative stress in diabetes mellitus has been shown to coexist with a reduction in the antioxidant status and glycation of proteins, inactivation of enzymes, and alteration in structural functions of collagen basement membrane (Boynes, 1991). Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes mellitus and may alleviate the diabetes mellitus as well as reduce its secondary complications.

Figure 1 describes the pathways for responses and signals during oxidative stress (Irshad and Chaudhuri, 2002).

In recent years, plants have been effectively tried on a variety of pathological conditions. Moreover, in the Indian traditional system of medicine, herbal remedies are prescribed for the treatment of many diseases including diabetes mellitus, etc. Many drugs commonly used today are of herbal origin. Some are made from plant extracts; others are made through transformation of chemicals found within them. While yet others are today synthesized from inorganic materials, but have their historical origins in research into the active compounds found in plants (Nirmala et al., 2009).

The World Health Organization (WHO) estimates that 4 billion people of the world population presently use herbal medicine for some aspect of primary healthcare (Pei, 2001). The global demand for herbal medicine is not only large but also growing. For the treatment of diabetes mellitus, Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas are found to control the blood sugar as long as they are regularly administered but they also produce a number of undesirable side effects (Mutalik et al., 2003)

Basella rubra, known as Malabar spinach or cyclone spinach, belongs to the family Basellaceae. It is a climbing perennial plant, particularly abundant in India, Malaysia and Philippines, but also seen throughout tropical Africa and tropical South America. Malabar spinach has thick tender stems with circular to ovate leaves that are alternate and short petioles. The leaves are thick, rugose, succulent and green to purple in color. The leaves are used in catarrhal affections and to hasten suppuration. Decoction of the root relieves bilious vomiting .It is also a good source of vitamins and minerals (Palada and Crossman, 1999).

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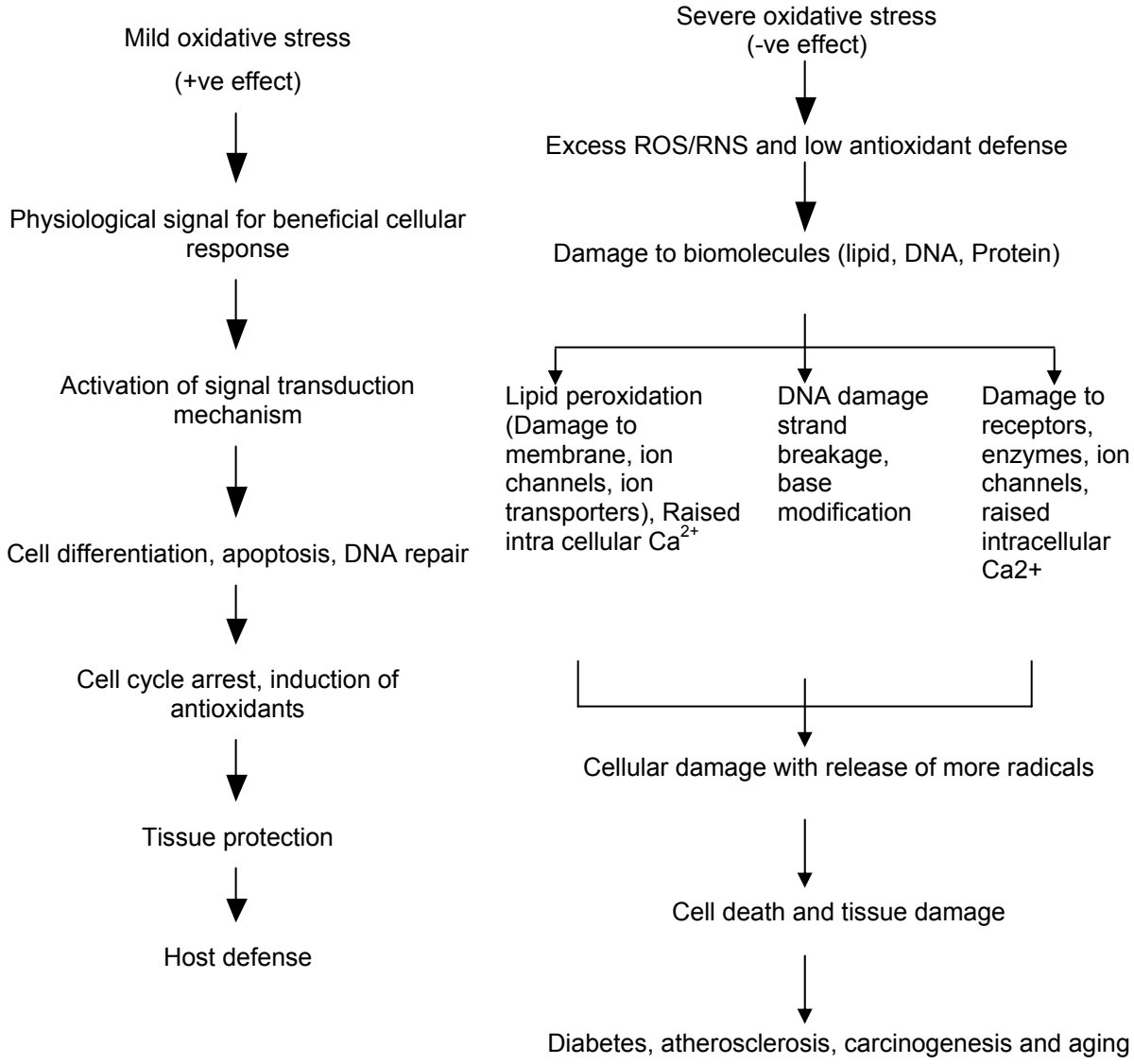

Figure 1. Pathways for responses and signals during oxidative stress. (Source: Irshad and Chaudhuri, 2002)

However, available literature shows that no experimental work has been carried out to verify the claims on the antidiabetic activity of *Basella rubra* and its relationship with its antioxidant properties. During diabetes mellitus persistent hyperglycemia causes an increased production of free radicals via auto oxidation of glucose and non enzymatic protein glycation, which may lead to disruption of cellular function and oxidative damage to membranes. Therefore, it was considered worthwhile to undertake this study to evaluate the antidiabetic activity of *Basella rubra* and its relationship with its antioxidant property.

2. MATERIALS NAD METHODS

Basella rubra young seedling was collected from the village area (Dindigul district, India) and raised in the university campus under normal climatic conditions. The plant was identified and authenticated (No. BSI/SC/5/21/04-05/Tech. 367) by Botanical Survey of India (BSI), Tamil Nadu Agriculture University (TNAU), Coimbatore, Tamil Nadu, India. Fresh leaves were collected for the study whenever required.

2.1 PREPARTION OF THE PLANT SAMPLE

Fresh leaves of *Basella rubra* (400mg) were collected and ground in a mortar with a pestle, with 10 ml purified water. The ground whole material was utilized for the experiments.

2.2 ANIMAL AND TREATMENT

Male albino rats were selected for the study. They were of the same age (2months) and weight (150-200gm). The rats were housed in polycarbonated clean cages under a 12/12 h normal light/dark cycle. The animals were fed with standard diet and water *ad libitum*. After keeping in the laboratory condition for a week for acclimatization the experiment was initiated. The total 24 male albino rats were categorized into following 4 groups, each group consisting of six rats.

1. Group I - Non Diabetic healthy control
2. Group II - Diabetic control, diabetes was induced in rats after 18 hours fasting by intraperitoneal administration of streptozotocin (60mg/kg body weight dissolved in10 mM citrate buffer with pH 4.5) (Archana et al., 2001). STZ injected animals exhibited massive glycosuria and hyperglycemia within few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration. A blood glucose level of 200mg/dl was considered as diabetic.
3. Group III - Non diabetic rats treated with pulp of *Basella rubra*.
4. Group IV - Diabetic rats treated with pulp of *Basella rubra.*

After induction, diabetes group III and IV were administered with the pulp of *Basella rubra* (400mg/kg body weight) daily for 30days. After 30days of treatment the rats were fasted overnight and scarified, the liver and blood sample was taken for the analysis of enzymatic and nonenzymatic antioxidants and glucose.

2.3 BIOCHEMICAL PARAMETERS

Glucose is primarily a compact energy store, and is the primary source of energy for body cells, fats and oils. So in the present study blood glucose was estimated in control as well as in diabetic animals by the method of Raghuramulu et al. (1983). The levels of reactive

oxygen species are controlled by antioxidant enzyme like SOD, CAT and GPx were measured by the method of Kakkar et al. (1984), Luck (1974) and Rotruck et al. (1984) respectively. The nonenzymatic scavengers such as reduced glutathione (GSH), Vitamin C and Vitamin E were measured by the methods of Moron et al. (1979), Omaye et al. (1979) Baker et al. (1951), respectively.

3. RESULTS

3.1 EFFECT OF BASELLA RUBRA ON BLOOD GLUCOSE AND ANTIOXIDANTS LEVEL

Glucose levels were found to be significantly increased after STZ administration, and there after decreased by administration of *Basella rubra*. Decrease in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ. Administration of *Basella rubra* extract produced a significant ($p<0.01$) decrease in the blood glucose as compared to diabetic control (Table 1).

Table 1. Blood glucose level in control, diabetic and treated rats

Groups	Treatments	Glucose (mg/dl)
I	Non-diabetic control	80.0 ± 3.65^{a}
II	Diabetic control	252.0 ± 4.16^{c}
III	Non-diabetic + *Basella rubra*	82.0 ± 4.76^{a}
IV	Diabetic + *Basella rubra*	110.1 ± 3.93^{b}
$CD_{(0.05)}$		5.46

Values are mean ± SD
Values with different superscripts differ significantly (P<0.05)

Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, muscular degeneration, diabetes mellitus, cancer etc are all contributed by oxidative damage. Hence the enzymatic and nonenzymatic antioxidant levels were measured in diabetic rats. The enzymatic antioxidant SOD, CAT and GPx levels were found to be lower in diabetic rats compared to that of the control rats (group I and III).These enzymatic antioxidant levels in diabetic rats treated with *B. rubra* significantly ($P<0.05$) increased to a level closer to the normal values (Table 2).

Table 3 shows the low levels of nonenzymatic antioxidant Vitamin C, E and reduced glutathione were observed in diabetic rats, when compared to that of control rats. The levels of these antioxidants were significantly increased in diabetic rats by treating with *B. rubra.*

Table 2. Effect of *Basella rubra* on superoxidedismutase, catalase and glutathioneperoxidase in liver of control, diabetic and treated rats

Groups	Treatments	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein/min)
I	Non-diabetic control	6.48 ± 1.26^{a}	75.0 ± 2.94^{a}	8.6 ± 0.39^{a}
II	Diabetic control	2.92 ± 0.11^{b}	34.98 ± 4.46^{c}	4.7 ± 0.48^{b}
III	Non-diabetic + *Basella rubra*	6.96 ± 0.99^{a}	73.90 ± 3.19^{a}	9.1 ± 1.95^{a}
IV	Diabetic + *Basella rubra*	6.08 ± 0.85^{a}	62.98 ± 2.96^{b}	8.7 ± 0.25^{a}
	$CD_{(0.05)}$	1.20	4.55	1.36

Values are mean ± SD;
Values with different superscripts are significantly different (P<0.05)
SOD (U/mg protein): amount of SOD that causes 50% reduction in the extent of NBT oxidation; CAT (U/mg protein): amount of enzyme that brings about a decrease in absorbance of 0.05 at 240 nm; GPx (U/mg protein): μmoles of NADH oxidized/mg protein/min

Table 3. Levels of vitamin E, C and reduced Glitathione in control, diabetic and treated rats

Groups	Treatments	Vitamin E (mg/g)	Vitamin C (μg/g)	Reduced glutathione (nanomoles/g tissue)
I	Non-diabetic control	0.37 ± 0.053^{a}	0.08 ± 0.028^{a}	8.67 ± 2.58^{a}
II	Diabetic control	0.18 ± 0.028^{c}	0.05 ± 0.029^{b}	5.03 ± 1.28^{b}
III	Non-diabetic + *Basella rubra*	0.53 ± 0.039^{b}	0.08 ± 0.031^{a}	8.77 ± 2.28^{a}
IV	Diabetic + *Basella rubra*	0.51 ± 0.030^{b}	0.07 ± 0.029^{a}	8.25 ± 2.30^{a}
	$CD_{(0.05)}$	0.11	0.04	2.85

Values are mean ± SD
Values with different superscripts are significantly different (P<0.05)

4. DISCUSSION

SOD, CAT and GPx constitute a mutually supportive team of defense against Reactive Oxygen Species (ROS). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of O_2^-. In hyperglycaemia, glucose undergoes auto oxidation and produces superoxide and it produce free radicals that in turn leads to lipid peroxidation in lipoproteins. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of Hydrogen Peroxide (H_2O_2) to water and oxygen and thus protects the body. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Sabu and Kuttan, 2004). In our study, decline in the activities of these enzymes in STZ induced animals and attainment of near normalcy in *B. rubra* treated rats indicates oxidative stress elicited by STZ had been nullified due to the effect of the extract. This observation perfectly agrees with those of Krishnakumar et al. (1999) who have demonstrated hypoglycaemic and antioxidant activity of *Salscia oblonga*

wall extract in STZ induced diabetic rats. Similar to the finding in this study, a decrease has been observed in the activities of SOD, CAT and GPx in some of the tissues of diabetic rats (Sekar and Govindasamy, 1990).

Glutathione (GSH) is a major non-protein thiol in living organism, which plays a central role in coordinating the body's antioxidant defense processes. Decreased GSH concentration contributes to the pathogenesis of complications associated with the diabetic state (Meister, 1983). Reduced glutathione, synthesized mainly in the liver, is an important nonenzymatic antioxidant in the antioxidative defence system. Kaplowitz and Ookhtens (1985) have remarked that the marked depletion of GSH observed in the tissue of alloxan diabetic mellitus rats, may be due to the utilization of this compound by two antioxidant enzymes GPx and GST as their substrate.

Vitamin C is an important water soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body. It readily oxidises to dehydroascarbic acid. Human beings have no ability to synthesis vitamin C due to mutation in the gene coded for L. gulonolactone oxidase, an enzyme required for biosynthesis of vitamin via the glucronic acid pathway. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins (Davie et al., 1992). Transport of vitamin C into cell is facilitated by insulin. Many diabetics do not have enough intracellular Vitamin C .Therefore, a relative Vitamin C deficiency exists in many diabetics despite adequate dietary consumption (Cunningham, 1991).

The most important antioxidant in the cell membrane is alpha tocopherol. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxyl damage (Metin, 2002). Increased level of alpha tocopherol found in the STZ diabetic rats as compared with control rats in our study may be due to the release of membrane bound alpha tocopherol from damaged cell membrane since it is water insoluble. The major function of vitamin E, that it protects the membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reactions. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. It may also potentiate the immune response (Wang and Quinn, 1999). Takenaka et al. (1991) have reported increased levels of alpha tocopherol in the liver of diabetic rats in spite of increased susceptibility to oxidation.

Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *B. rubra* can rightly be mentioned as a plant of considerable interest.

5. CONCLUSION

The result of the present study showed that *B. rubra* brings back the blood glucose and also increase the antioxidant level in experimental rats. From the above results it is shown that it has (*B. rubra*) hypoglycemic activity. Hypoglycemic action of the herbal plant (*B. rubra)* in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscle. It may prevent the hepatic injury and pancreas and suppressing the oxidative stress associated with diabetes. Although

the exact chemical compounds responsible for the hypoglycemic effects of *B. rubra* still remain speculative, experimental evidence obtained from this study indicates that *B. rubra* has an antioxidant activity and also possess hypoglycemic property.

REFERENCES

Archana, S., Rashmi, N., Khemani, L.D. (2001). Hypoglycemic effect of Hibiscus rosasinensis L. leaf extract in glucose and streptozotocin-induced hypoglycemic rats. Ind. J. Exp. Biol., 39, 284-286.

Baker, H., Frank, O., Angelis, B., Feingold, S. (1951). Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Rep. Inv., 21, 531-536.

Boynes, J.W. (1991). Role of oxidative stress in development of complication in diabetes. Dabetes, 40, 405-411.

Cunningham, J. (1991). Reduced mononuclear leucocyte ascorbic acid content in adults with insulin–dependent diabetes mellitus consuming adequate dietary Vitamin C. Metabolism, 40,140-149.

Davie, S.J., Gould, B.J., Yudkim, J.S. (1992). Effect of Vitamin C on glycosylatin of protein. Diabetes, 41, 167-73.

Irshad, M., Chaudhuri, P.S. (2002). Oxidant-antioxidant system: Role and significance in human body. Ind. J. Exp. Biol., 40, 1233-1239.

Kakkar, P., Das, B., Viswanathan, P.N. (1984). A modified spectrophotometric assay of SOD. Ind. J. Biochem. Biophys., 21,130-132.

Krishnakumar, K., Augusti, K.T., Vijayammal, P.L. (1999). Hypoglycemic and antioxidant activity of Saacia oblonga wall extract in streptozotocin–induced diabetic rats. Ind. J. Physiol. Pharmacol., 43(3), 510.

Kaplowitz, N., Ookhtens, M., Aw, T.V. (1985). The regulation of hepatic glutathione. Annu. Rev. Pharmacol. Toxicol., 25, 715-744.

Luck, H. (1974). In: Methods in enzymatic analysis -2(Ed.Bergmeyer), Academic Press, New York, 885.

Mutalik, S., Sulochana, B., Chetana, M., Udapa, N., Devi, V.P. (2003). Preliminary studies on acute and subacute toxicity of an antidiabetic herbal preparation – Dianex. 41, 316-320.

Moron, M.S., De Pierre, J.N., Mannervik, V. (1979). Levels of glutathione, glutathione reductase, glutathione –S- transferase in rat lung and liver. Biochem. Biophys. Acta, 582, 67-68.

Meister, A. (1983). Selective modification of glutathione metabolism. Sci., 20, 472-477.

Metin,G., Atukeren, P., Gumustas, K.M., Belce, A., Kayserilioglu, A. (2002). The effect of Vitamin E treatment on oxidative stress generated in trained rats. J. Exp. Med., 198, 47-53.

Nirmala, A., Saroja, S., Hannah, R. V. (2009). Effect of *Basella rubra* in diabetic rats. J. Medicinal Food Plants, 1(1),10-15.

Omaya, S.T., Turbul, T.D., Sauberlich, H.C. (1979). Selected method for the determination of ascorbic acid in animal cell, tissue and fluid. Mc. Cromic, DB Write DL (eds) Methods of Enymology, Academic Press, New York ,62,3-11.

Pei, S. (2001). Ethnobotanical approaches of traditional medicine studies: some experience from Asia. Pharmaceut. Botany, 39, 74-79.

Palada, M.C., Crossman, S.M.A. (1998). Planting density affects growth and yield of bush Okra. Proc. Caribben Food Crops Soc., 34.

Raghuramulu, N., Nair, M.K., Kalyanasundram, S.A. (1983). Manual of laboratory techniques, I edition, National Institute of Nutrition, KMR, Hyderabad, 31-32.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B. (1984). Selenium: Biochemical roles as a component of glutathione peroxidase. Sci., 179, 588-590.

Sabu, C.M., Kuttan, R. (2004). Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties. Ind. J. Physiol. Pharmacol., 48(1), 81-88.

Sekar, N., Govindasamy, S. (1990). Insulin mimetic role vanadate on plasma membrane insulin receptor. J. Biosci., 15, 115.

Takenaka, Y., Miki, M., Yasudha, H., Mino, M. (1991). The effect of alpha tocopherol as an antioxidant of membrane protein thiols induced by free radicals generated in different sites. Arch. Biochem. Biophys., 285,344-50.

Wilson, R.L. (1998). Free radicals and tissue damage, Mechanistic evidence from radiation studies. In: Biochemical mechanisms of liver injury, New York, Academic Press, 123-125.

Wang, X., Quinn, P. (1999). Vitamin E and its function in membranes. Prog. Lipid Res., 38(4), 309 – 336.