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ANTIOXIDANT ENHANCING PROPERTY OF CURRY LEAF POWDER (*MURRAYA KOENIGII*) IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

People with diabetes have greater antioxidant requirements due to increased production of free radicals in hyperglycemia. Decreased activity of the antioxidant enzymes may increase the susceptibility of diabetic patients to oxidative injury. Carotenoids and Vitamin C present in curry leaves possess antioxidant property, which helps to neutralize and counteract the deleterious free radicals. This study evaluates the antioxidant potential of *Murraya koeinigii* (curry leaf powder) in Type 2 Diabetes Mellitus. Twenty male Type 2 diabetic patients, 50-65 years formed the samples. The Diabetic Experimental Group (DEG) was supplemented with 15 g of curry leaf powder for a period of 30 days. Antioxidant parameters such as SOD, GSH-Px, Vitamin E and Vitamin C were analyzed. A significant change in antioxidant levels in the DEG was observed. Curry leaf powder had the property to increase the antioxidant levels which, if consumed regularly can delay complications associated with diabetes mellitus.

KEY WORDS: **Type 2 Diabetes, *Murraya koenigii*, antioxidants**

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INTRODUCTION

Oxidative stress refers to a serious imbalance between reactive species (RS) production and antioxidant defenses. Growing evidences indicate that oxidative stress is increased in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant system. People with diabetes have greater antioxidant requirements because of increased production of free radicals in hyperglycemia.[1] Diabetic patients have significant defects of antioxidant protections and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications.[2] The ability of the cells or tissues to withstand oxidative stress is largely dependent on the efficiency of the overall antioxidant defense system to scavenge excess RS.[3] Antioxidant defense system includes enzymic antioxidant molecules such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) and non-enzymatic components such as Vitamin A, Vitamin C and Vitamin E. Evidence of oxidative stress can be provided by measuring markers of this stress or antioxidant molecules[1]. *Murraya koenigii* is a tropical to subtropical tree in the family rutaceae, which is native to India. The plant is highly valued for its leaves as an important ingredient in Indian cuisine. Carotenoids and Vitamin C present in curry leaves possess antioxidant property and it helps to neutralize and counteract the deleterious free radicals.[4] The *Murraya koeinigii* leaves which has the richest source of carbazole alkaloids such as koenigine, mahanimbine, muconicine is reported to have anti-diabetic activities. Curry leaves also contain higher amounts of chlorophyll. Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals.[5] Hence this study was undertaken to analyse the Antioxidant effects of Curry Leaves (*Murraya koeinigii*) in Type 2 Diabetes Mellitus.

METHODS AND MATERIALS

Sample selection

The study subjects were selected based on the following criteria: 50-65 years of age, male, those with diagnosed Type 2 diabetes, taking only oral hypoglycemic drugs, taking regular food without any other herbal supplementation, non-smokers, non-alcoholics, no other history of chronic disease and those willing to participate in the study. Based on the selection criteria, 20 Type 2 Diabetic patients were purposively selected from the Community Health Center, General Hospital of Karikalampakkam, Puducherry. They were grouped into Diabetic Control Group (DCG n=10) and Diabetic Experimental Group (DEG n=10).

Supplementation with curry leaf powder

Curry leaves were cleaned and dried at room temperature. It was further dried using micro oven for 3 minutes and finely powdered. 100g of fresh curry leaves gave 40g of powder. The powder was prepared with utmost care and packed in zip lock polythene packets of 15g each and distributed to the study subjects. The 15g powder provided 1.5 g of carbohydrate, 1.3 g of fiber, 1.02 mg µg of ß – Carotene, 94.29 mg of total chlorophyll and 17.58 mg of Vitamin C. The powders were freshly prepared once in three days and given to the Diabetic Experimental Group for a period of 30 days along with regular food and medication. They were asked to consume the entire powder during lunch. The Diabetic Control Group did not receive any supplementation of curry leaf powder and were taking regular food and medication.

Evaluation of antioxidant parameters

Antioxidant parameters such as Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), Vitamin C and Vitamin E levels of the DEG was analyzed before and after supplementation with curry leaf powder for 30 days. The same parameters were recorded for the DCG before and after the same period.

RESULTS AND DISCUSSION

1. Comparison of oxidative stress among non-diabetics and diabetics

Oxidative stress is found to be prevalent in a system where the free radical production is increased and/or the antioxidant mechanisms are impaired. Decreased activity of the antioxidant enzymes may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supplies may help to prevent clinical complications of diabetes mellitus.[6] The human body defends itself naturally against free radicals, by producing endogenous antioxidant enzymes like SOD, GSHPx and Catalase. With ageing, the body decreases its production of these antioxidant enzymes and the harmful action of free radicals becomes incisive. The non enzymatic antioxidant, like vitamin A, vitamin C and vitamin E are derived from natural sources by dietary intake. Any disease increases the oxidative stress because of a decrease in the antioxidant molecules. Diabetes is one such condition where a decrease in antioxidant levels has been reported. The prevalence of more number of ailments further impairs the protective antioxidant mechanisms leading to degeneration in body functions.[1] In order to compare the antioxidant status of healthy individuals and those with ailments, a comparison was done among healthy, non- diabetic individuals (Group I: n=10), those with diabetes only (Group II: n=20) and those with diabetes and hypertension (Group III: n= 7). Table 1 and Figure 1 presents the comparison of the antioxidant levels

Table 1
Comparison of Oxidative Stress among Non-Diabetics and Diabetics

Variables	Non-Diabetics Group I (n=10) Mean ± SD	Diabetics Group II (n=20) Mean ± SD	Diabetics with Hypertension Group III (n=7) Mean ± SD	F value	p value
Enzymatic Antioxidants					
SOD (U/l)	533 ± 79.9	452.8 ± 60.7	438.1 ± 46.5	10.710	0.0001**
GSHPx (U/l)	69 ± 7.8	55.1 ± 6.2	54.1 ± 21.7	10.097	0.0001**
Non-Enzymatic Antioxidants					
Vitamin C (mg/dl)	10.3 ± 1.5	9.2 ± 1.9	7.7 ± 1.3	14.897	0.0000**
Vitamin E (mg/dl)	12.9 ± 1.0	11.9 ± 2.6	9.8 ± 1.6	13.718	0.0000**

Significant at p < 0.01

From the above table, it is evident that there is a significant difference in the antioxidant activity of SOD, GSHPx, Vitamin C and Vitamin E between the non-diabetic, diabetic group and diabetic with hypertension group. This can also be observed by the reduction in the mean values between the three groups. The antioxidant levels were less in those who had diabetes and those with diabetes and hypertension compared to the non-diabetics. This clearly indicates that the oxidative stress increased with an increase in the disorders of the individuals. The antioxidant levels were the least in Group III, which could be due the additional metabolic stress due to hypertension.

Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes. Serum SOD activity had significantly decreased in diabetic subjects and in subjects with diabetes and hypertension compared to the healthy non-diabetic subjects. At the molecular level, the auto oxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD and leads to

accumulation of hydrogen peroxide which may be one of the explanations for decreased activity of SOD in Type II diabetic patients.[7] However, there was no significant change in SOD activity between Group II and Group III subjects, though a decrease in the mean SOD levels can be seen.

Glutathione peroxidase (GSHPx)

Glutathione peroxidase (GSHPx) is an enzyme that catalyses the reduction of hydroxyperoxides by glutathione. Its main function is to protect against the damaging effect of endogenously formed hydroxyperoxides (www.ebi.ac.uk). The serum GSHPx activity had significantly decreased in the diabetic group compared to the healthy non-diabetic group. Similar results have been observed in studies[8, 9] which reported a significant decrease of GSHPx activity in diabetic subjects compared with control subjects. They attributed this decrease to a decline in blood glutathione content in those diabetics, since GSH is a substrate and cofactor for this enzyme. Therefore, low GSH content indicate low GSHPx activity, which may produce increased oxidative stress. Low levels of GPx3 are also associated with the development of vascular disease. Individuals with both low HDL and GPx3 activity are at markedly increased risk for death from CVD.[10]

Vitamin C

Vitamin C is an antioxidant, which blocks some of the damage caused by free radicals to the DNA.It is available to the body only through diet and is not endogenously synthesized.[11] Patients with diabetes or the metabolic syndrome have low levels of the antioxidant vitamin C.[12] Being a naturally available antioxidant, it was found that the serum vitamin C level had significantly decreased in Group II subjects with diabetes compared to the Group I healthy non-diabetic individuals.

Vitamin E

Vitamin E is an antioxidant, a substance that helps prevent damage to the body's cells. (www.umm.edu). Vitamin E had significantly decreased in Group II who had diabetes compared to the non-diabetic group. Sufficient intake of antioxidants plays an important role in protection against Type II diabetes. However, little epidemiological evidence is available on the role of dietary antioxidant intake in the prevention of Type II diabetes. Vitamin E intake was significantly associated with a reduced risk of Type II diabetes. Montonen et al have reported that the development of Type II diabetes may be reduced by the intake of antioxidants in the diet.[13]

2. Antioxidant effect of curry leaf powder

The effect of curry leaves in reducing oxidative stress by increasing the antioxidant levels was analysed and is presented in Table 2 and Figures 2, 3, 4 and 5.

Table 2
Antioxidant Effect of curry leaf powder

Antioxidant Parameters	Initial Mean ± S.D	Final Mean ± S.D	Difference	t value	p value
Enzymatic Antioxidant					
SOD (U/l)					
DCG	473.6 ± 54.4	481.2 ± 44.7	+7.6	0.376	0.715
DEG	445.6 ± 59.2	488.2 ± 41.8	+42.6	3.356**	0.008
GSHPx (U/l)					
DCG	56.7 ± 4.6	59.4 ± 9	+2.7	0.858	0.412
DEG	50.4 ± 4.9	56.5 ± 7.7	+6.1	2.161	0.058
Non-Enzymatic Antioxidant					
Vitamin C (mg/l)					
DCG	8.8 ± 1.9	9.2 ± 2.1	+0.4	4.263**	0.002
DEG	9.4 ± 1.9	9.7 ± 1.6	+0.3	1.909**	0.008
Vitamin E (mg/l)					
DCG	12.7 ± 3.4	14.44 ± 3.9	+1.7	1.693	0.124
DEG	11.8 ± 3	14.47 ± 2.9	+2.6	3.829**	0.004

Significant at p < 0.01

Antioxidant levels of the Diabetic Control Group (DCG) before and after the supplementation period

From the table 2 it can be observed that there was no significant change in the antioxidant levels in the Diabetic Control Group after the 30 day period. This implies that the antioxidant levels of those in the control group were more or less the same. They had not received any supplementation and were following their regular diet and medicine regimen.

Antioxidant levels of the Diabetic Experimental Group (DEG) before and after supplementation with curry leaf powder

It is evident from Table 2 that there is a significant difference in the antioxidant activity of SOD, Vitamin C and Vitamin E in the Diabetic Experimental Group who consumed curry leaf powder. Consuming curry leaf powder regularly for a period of 30 days had increased these antioxidant levels. Similar results were observed in a study conducted by Sadhana etal which suggests that nutritive antioxidant compounds in M. koenigii play a pivotal role in the therapeutics of hepatotoxicity by increasing the body's natural antioxidant defenses with depletion in the ethanol-induced oxidative stress and reduction in the elevated levels of liver enzymes.[14] Curry leaves contain carbohydrates, proteins, fiber, minerals, chlorophyll, vitamin B, vitamin C, vitamin E, nicotinic acid and plant sterols. It is rich in vitamin A, calcium, phosphorus, magnesium, iron and oxalates. Curry leaves contain essential minerals such as iron, copper and zinc that aid in maintaining normal glucose level in the blood. It also contains many phenols, flavonols, aminoacids and carbazole alkaloids. The Murraya species have richest source of carbazole alkaloids.[15] Phenols and flavonols have protective action against oxidative damage of tissues. The lower strength of the O-H bond present in phenolics corresponds to a higher scavenging activity. A high correlation between antioxidant capacities and total phenolic contents indicates that phenolic compounds were a major contributor of antioxidant activity of curry leaves and provides evidence on the potential health benefits of these plants.[5] Carbazole alkaloids such as koenigine, mahanimbine, muconicine extracted from curry leaves have been found to demonstrate anticancer properties and antioxidant properties. Curry leaves contain higher amount of chlorophyll. Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals[5]. It efficiently delivers magnesium and helps the blood in carrying the much needed oxygen to all cells and tissues. Along with vitamins such as A, C and E, chlorophyll has been seen to help neutralize free radicals that do damage to healthy cells.[16] Individual who suffers from diabetes cannot manage the sugar levels in the blood. The pancreatic alpha-amylase is an enzyme that aids in the disintegration of starch intake through all types of foods one consumes. Pancreatic alpha-amylase is a digestive enzyme which helps in the breakdown of starches to glucose (the fundamental block of carbohydrates or sugars). Curry leaves helps to stop the production of this enzyme which helps in the sugar or glucose not being released into the blood. By reducing the availability of the pancreatic alpha-amylase enzyme, the leaves are able to inhibit the conversion of starches to sugar, which can help treat diabetes effectively (www.innovateus.net). Curry leaves contain high amount of fiber. Researchers have found that dietary fiber reduces a diabetic's need for insulin, improves blood-glucose control, lowers blood cholesterol and fat levels, and helps with weight loss.[17] Dietary fiber helps to slow the release of sugar into the bloodstream, thus helping to keep the blood sugar levels normal. *Murraya koenigii* is one of the most widely acclaimed remedies for the treatment of diabetes. Recent studies have revealed that consuming curry leaves helps in controlling the intensity of diabetes in Type II diabetes mellitus.[18] Table 3 gives the nutrient analysis of the curry leaf powder given for supplementation

Table 3
Nutrient Analysis of the Curry Leaf Powder given for Supplementation

Nutrients	Value Obtained (Per 100g)	Value Obtained for quantity supplemented (Per 15g)
Carbohydrate	10.1g	1.5 g
Crude Fiber*	8.65 g	1.3 g
β – Carotene**	6.828 mg	1.024 mg
Total Chlorophyll**	628.6 mg	94.29 mg
Chlorophyll a**	326.8 mg	49.02 mg
Chlorophyll b**	295.0 mg	44.25 mg
Vitamin C**	117.2 mg	17.58 mg

Hypoglycemic Property ** Antioxidant Property

Let Your Food be Your Medicine and Your Medicine be Your Food - Hippocrates

Figure 1: Comparison of Mean Serum Antioxidant Levels

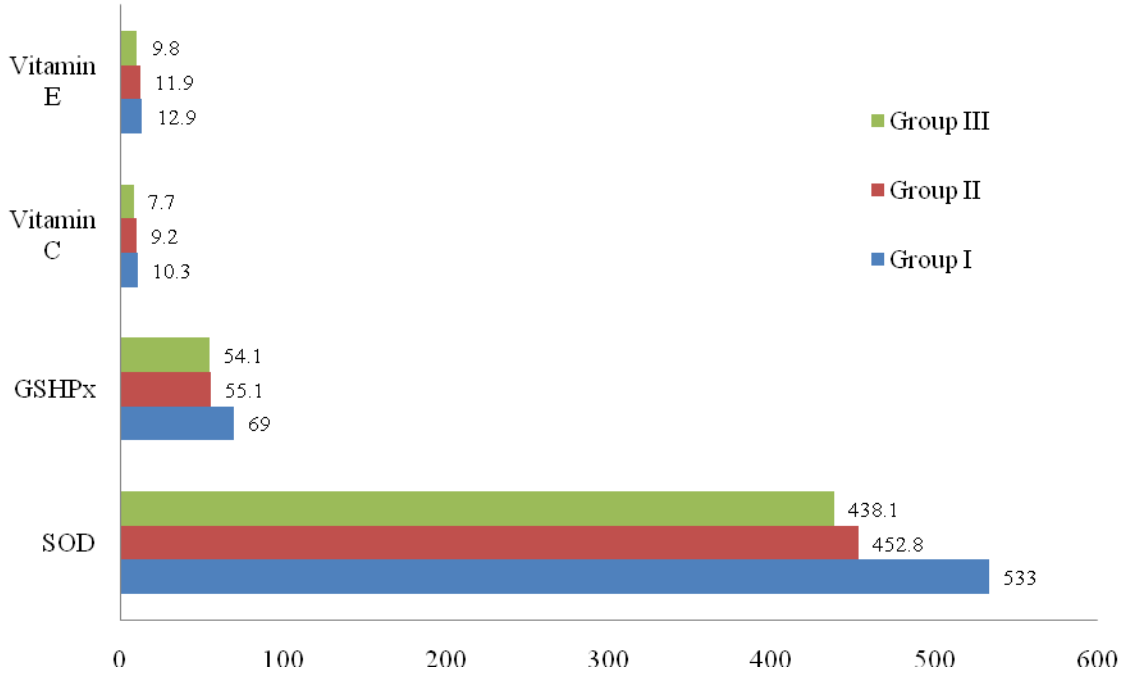


Figure 2: Change in Mean Serum SOD Levels

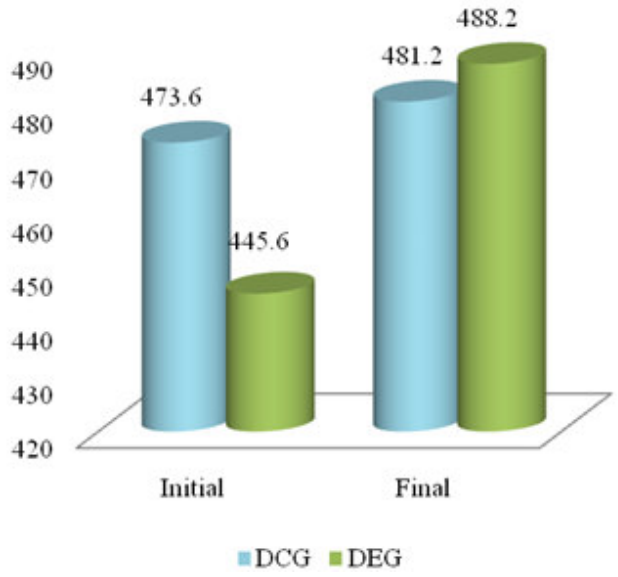


Figure 3: Change in Mean Serum GSHPx Levels

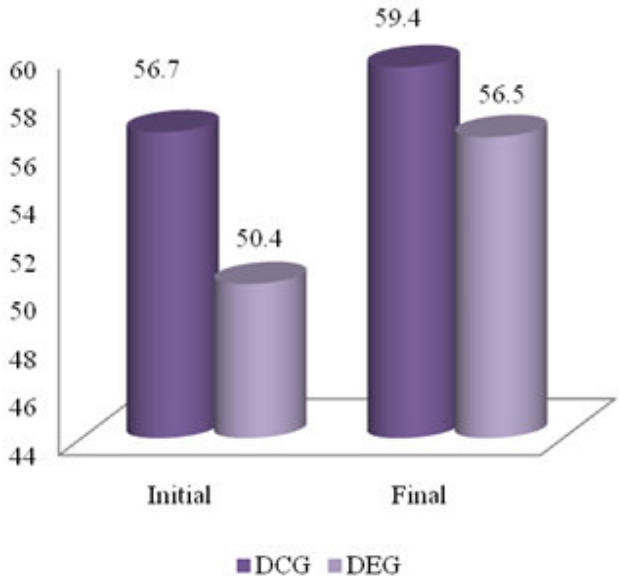


Figure 4: Change in Mean Serum Vitamin C Levels

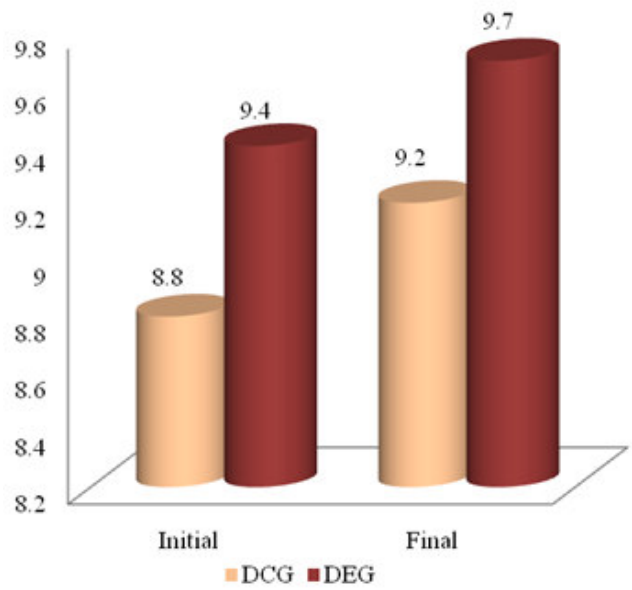
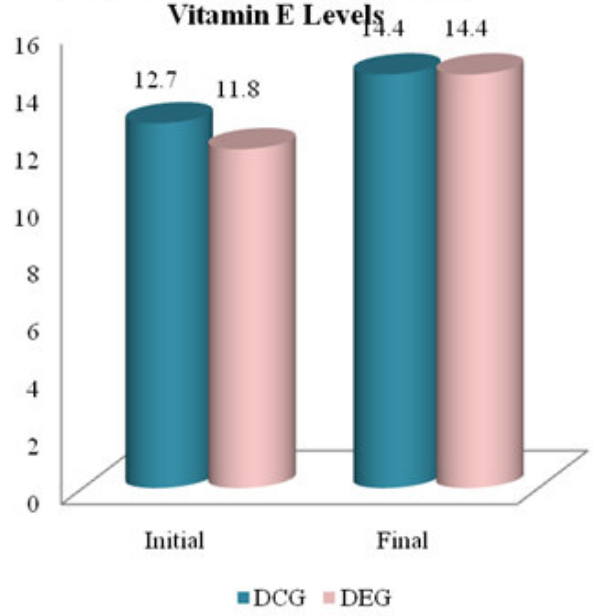


Figure 5: Change in Mean Serum Vitamin E Levels



CONCLUSION

The present study substantiates that curry leaf powder has antioxidant properties by elevating the antioxidant levels in the diabetic subjects. Curry leaf is also known for its anti-diabetic, antimicrobial, anti-inflammatory, hepatoprotective and antihypercholesterolemic properties. Curry leaves are easily available and cost effective and can be regularly incorporated in the diet of the diabetics without side effects which makes it a wholesome ingredient for regular consumption. Curry leaf with its various biological constituents can be used effectively to treat diabetes.

REFERENCES

1. Ramakrishna Vadde, Rama Jailkhani. Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. 2:22 doi: 10.1186/ 1746- 1596-2-22. (2007)
2. Mosaad A. Abou-Seif, Adb-Allah Youssef. Evaluation of some biochemical changes in diabetic patients. Clinica Chimica Acta 346 (2004) 161 – 170. (2004)
3. Halliwell B, Gutteridge J.M.C. Free Radicals in Biology and Medicine. 4th Edn. Oxford University Press, ISBN: ISBN-13: 978-0198568698 . (2007)
4. Johnson E J, Hammond B R, Yeum K J, Qin J, Wang X D, Castaneda C, Snodderly D M, Roussell R M. Relation among serum and tissue concentration of lutein and zeaxanthin and macular pigment density", American Journal of Clinical Nutrition. 71:1555-1562. (2000)
5. Garg, Aditya Muley, Nishtha Khare, Thankamani Marar. Comparative Analysis of Phytochemical profile and antioxidant activity of some Indian culinary herbs. Research Journal of Pharmaceutical. Biological and Chemical Sciences. volume3/issue 3. 845-854. (2012)
6. Shinde N Sarita, Vithal N Dhadke, Adinath N Suryakar. Evaluation of oxidative stress in type 2 diabetes mellitus and follow-up along with vitamin E supplementation. Ind J Clin Biochem. 26(1):74-77. (2011)
7. Nobar R, Pour R. Total antioxidant capacity, SOD and GSH in diabetic patients, Med J Islam Acad Sci. 12(4): 109-14. (1999)
8. Jos S K, Rybak M, Patin P H, Robert J J, Boitard C, Thevenin R. Antioxidants and Insulin Dependent Diabetes Mellitus and adolescents. 16: 498-503. (1990)
9. Dominguez C, Ruiz E, Gussinye M, Carrascisa A. Oxidative stress at onset and in early stages of Type 1 Diabetes in children and adolescents. Diabetes Care: 21: 1736-42 (1998)
10. Buijsse B, Lee D-H, Steffen L, Erickson RR, Luepker RV, et al. Low Serum Glutathione Peroxidase Activity Is Associated with Increased Cardiovascular Mortality in Individuals with Low HDLc's. PLoS ONE 7(6): e38901. doi:10.1371/journal.pone.0038901 (2012)
11. Mario C. De Tullio. The Mystery of Vitamin C. Nature Education 3(9):48. (2010)
12. Fadupin G T, Akpoghor A V, Okunade K A. A comparative study of serum ascorbic acid level in people with and without type 2 diabetes in Ibadan, African Journal of Medicine and Medical Sciences, Vol.36, no.4, 335-339. (2007)
13. Jukka Montonen, Paul Knekt, Ritva Järvinen, Antti Reunanen. Dietary Antioxidant Intake and Risk of Type 2 Diabetes. Diabetes Care, doi:10.2337/diacare.27.2.362 vol. 27 no. 2 362-366. (2004)
14. Sadhana Sathaye, Poornima D amin, Vinam B Mehta, Vijay B Zala, Ramesh D Kulkarni, Harpreet Kaur and Roopali G Redkar. Hepatoprotective Activity of Murraya koeinigii against ethanol induced liver toxicity model in experimental animals. International Journal of Pharma and Bio Sciences. ISSN 0975-6299 Vol 3/Issue 1/Jan – Mar 2012.
15. Bonde S D, Nemade L S, Patel M S, Patel A. Murraya koenigii (Curry Leaf): Ethnobotany, Phytochemistry and Pharmacology. International Journal of Pharmaceutical and Phytopharmacological Research. 1(1): 23-27. (2011)
16. Kizhedath, Arathi and Suneetha V. Estimation of chlorophyll content in common household medicinal leaves and their utilization to avail health benefits of chlorophyll. Journal of Pharmacy Research. 4(5). 1412-1413. (2011)
17. Iyer U M and Mani U V. Plant Food Hum. Nutri. 40(4). 275-282. (1990)
18. Taraporewala, Azmin. Curry leaves for Diabetes, Buzzle.com (2012).