INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

THE EFFECT OF QUERCETIN ON BLOOD GLUCOSE LEVELS OF NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC (TYPE I & TYPE II) RATS

Ketan Hatware* and A. Annapurna

A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

This study was conducted to find out the effect of Quercetin on normal as well as diabetic (Type I & Type II) rats.Wistar strain rats of either sex weighing 150 g -250 g were used for the study. Quercetin was given to normal as well as diabetic (Type I & Type II) rats by oral and intraperitoneal route of administration. Streptozotocin (40 mg/kg IV) was used to induce type I diabetes while Streptozotocin (65 mg/kg IV) and Nicotinamide (150 mg/kg IP ten min prior to dose of Streptozotocin) were used to induce type II diabetes in rats. Rats were fasted for 12 hrs and test (Quercetin) drug was administered by 25 mg/kg oral as well as 10 mg/kg intraperitoneal route and blood glucose levels were estimated by GOD POD method using semi-autoanalyser (Screen Master 3000) at 0 hr, 2nd hr, 4th hr, 6thhr, 8thhr, & 12th hr time intervals. Quercetin has shown significant blood glucose level reduction in normal as well as diabetic (Type I & Type II) rats. The peak reduction in blood glucose level was observed at 8th hr. Hence it was concluded that the Quercetin is having the potential to use in the field of diabetes.

Keywords: Quercetin, Streptozotocin (STZ), Blood glucose, Diabetes.

INTRODUCTION

Diabetes mellitus is a metabolic disorder in the endocrine system causing hyperglycemia. Diabetes affects about 5% of the global population[1] and management of diabetes without any side effects is still a challenge to the medical system[2]. In India, the prevalence rate of diabetes is estimated to be 1-5%. Diabetes is becoming the third “killer” of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, morbidity and mortality. The cause of diabetes is a mystery, although both genetic and patient related factors such as obesity and lack of exercise appear to play a role. Ethnic and racial differences have been found in heterogeneous populations within the same area. As a rule, incidence is highest in Scandinavian countries, intermediate in the US, Spain, and Israel, and lowest in Asian and most Latin American countries. Most researchers believe that, in the presence of agenetic predisposition, something in the environment triggers the development of diabetes. With a long course and serious complications often resulting in high death rate, the treatment of this disorder takes three main forms: (I) Diet and exercise (II) Insulin replacement therapy and (III) the use of oral hypoglycemic agents. Currently available synthetic antidiabetic agents like sulfonylureas, biguanides, α-glucosidase inhibitors etc.besides being expensive produce serious side effects. Further their use is not safe during pregnancy. Apart from currently available therapy, herbal medicines recommended for treatment of diabetes throughout the world. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost[3]. Thus due to an increase in demand by patients to use natural products with antidiabetic activity, investigations on hypoglycemic agents derived from medicinal plants have gained popularity in recent years. Laboratories are conducting research on these medicinal plants in a scientific manner for the development of alternative drugs and strategies for better management of diabetes. Bioflavonoids are the polyphenolic

compounds that are widely found in plants, fruits and vegetables[4]. These are well known antioxidants having various therapeutic activities such as anti-inflammatory, antioxidant, antiallergic, anticarcinogenic, antiviral, antibacterial and antifungal effects[5]. Some bioflavonoids has shown antihyperglycemic activity for example procyanidin a bioflavonod has insulin like effects in insulin sensitive cells that could help to explain their antihyperglycemic effect in vivo[6].*Ipomoea batatas* leaf which contains flavone, is used as antidiabetic drug in streptozotocin induced diabetic rats[7]. *Ginkgo biloba*is used as antidiabetic drug in streptozotocin induced diabetic rats, ginkgo biloba contains bioflavonoids such as quercetin[8]. Quercetin is one of the most important bioflavonoid having great therapeutic potential. Quercetin scavenges oxygen radicals,[9] inhibits xanthine oxidase and inhibits lipid peroxidation invitro[10,11]. As another indicator of its antioxidant effects, quercetin inhibits oxidation of LDL cholesterol invitro, probably by inhibiting LDL oxidation itself, by de protecting vitamin E in LDL from being oxidized or by regenerating oxidized vitamin E[12]. By itself and paired with ascorbic acid, quercetin reduced the incidence of oxidative damage to neurovasculature structure in skin, and inhibited damage to neurons caused by experimental glutathione depletion[13]. Animal studies have shown quercetin to be protective of gastric ulcer, action caused by ethanol, probably by inhibiting lipid peroxidation of gastric cells and/or by inhibition of gastric acid secretion[14,15]. Quercetin has been investigated in a number of animal models and human cancer cell lines and has been found to have anti proliferative effects. It may also increase the effectiveness of chemotherapeutic agents[16]. The previous studies shown that the quercetin is having the antidiabeticactivityin streptozotocin induced diabetic rats**.** It is concluded that quercetin, a flavonoid with antioxidant properties brings about the regeneration of the pancreatic islets and probably increases insulin release in streptozocin-induced diabetic rats; thus exerting its beneficial antidiabetic effects[17].
Asmentioned above some bioflavonoids are useful in the diabetes mellitus and quercetin a bioflavonoid has good therapeutic potential in many pathological conditions, our interest is to evaluate the effect of quercetin on blood glucose levels in normal and diabetic (Type I & Type II) rats. With this objective, in the present study, experimental protocol, results and discussion are presented as follows;

MATERIALS AND METHODS

Animals used in study

Wistar albino rats of either sex were procured from Mahaveer Enterprises, Hyderabad, India. The animals were maintained on a 12 hour light and 12hour dark cycle. They were fed, ad libitum regular grain chow (Rayans Biotechnologies Pvt. Ltd., Hyderabad). Diet containing 56% grain derived carbohydrate, 21% protein, 6.7% moisture, 3.58% total oil, 2.58% dietary fiber, 5.5% cellulose, 0.8% calcium, 0.6% phosphrous, 0.3 % sodium chloride. The animal housing and handling were in accordance with CPCSCA guidelines. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

Chemicals used in study

Quercetin (Sigma chemicals Ltd., USA)**,**Streptozotocin (Sigma chemicals Ltd., USA)**,**Nicotinamide (Sigma chemicals Ltd., USA)**,**Citrate buffer pH 4.5 (I.P). Unless otherwise specified all the chemicals and reagents used are of analytical grade.

Method of induction of diabetes

Streptozotocin was dissolved in freshly prepared citrate buffer with P^H 4.5 and then the solution was injected within 5 min. For induction of type I diabetes, 40 mg/kg of STZ was given through tail vein of the rat. For induction of type II diabetes, 150 mg/kg Nicotinamide IP, then 65 mg/kg STZ was given through tail vein of the rat. Blood sample was collected from the retro orbital plexus of rats after 48 hrs and glucose levels were estimated.Rats with glucose level above 200 mg/dl were used for the study.

Estimation of blood glucose level in rats

In this study the enzymatic; glucose oxidase-perixodase (GOD – POD) method[18] was used.

Preparation of drug solution

The quercetin was suspended in the 1% sodium carboxy methyl cellulose (CMC) mucilage with continuous trituration.

Procedure (Experimental protocol)

Albino Wistar strain rats of either sex weighing 150g-250g were used for the study. The rats were kept on fasting for 12 hours before the experiment. The test drug (quercetin) was administered by oral (25 mg/kg) as well as intraperitoneal (10 mg/kg) route, in following groups; (each group consist of 6 animals).
Group I: Normal control rats treated with 1% sodium CMC 1ml orally.

GroupII:Normal control rats treated with 1% sodium CMC 1ml IP.
Group III: Normal rats treated with quercetin 25mg/kg orally.
Group IV: Normal rats treated with quercetin 10 mg/kg IP.
Group V: Type I diabetic control rats treated with 1% sodium CMC 1ml orally.
Group VI: Type I diabetic control rats treated with 1% sodium CMC 1ml IP.
Group VII: Type I diabetic rats treated with quercetin 25mg/kg orally.
Group VIII: Type I diabetic rats treated with quercetin 10 mg/kg IP.
Group IX: Type II diabetic control rats treated with 1% sodium CMC 1ml orally.
Group X: Type II diabetic control rats treated with 1% Sodium CMC 1ml IP.
Group XI: Type II diabetic rats treated with Quercetin 25mg/kg orally.
Group XII: Type II Diabetic rats treated with Quercetin 10 mg/kg IP
The rats were kept on fasting for 12 hours before the experiment. The blood samples were collected from the retro orbital plexus of rats at 0 hr, 2nd hr, 4th hr, 6thhr, 8thhr, & 12th hr time intervals. After collection of blood sample, the serum was separated by centrifuge at 3000 rpm for 10 min. The serum glucose estimation was done with the Screen Master 3000 (Auto Analyzer for Biochemical parameters).

Statistical analysis

The data was statistically analyzed by one-wayANOVA followed by Dunnett multiple comparisontest with equal sample size. The difference wasconsidered significant when $p<0.001$. All the values were expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

As per the observations in normal rats, quercetin significantly reduced the serum glucose levels with both oral (25 mg/kg) as well asintraperitoneal (10mg/kg)administration. This indicates that quercetin has produced hypoglycemic activity. This finding was a contradictory to the previous study[17] in which quercetin had no effect on plasma glucose levels in normal rats. However, the probable mechanism for hypoglycemic activity is uncertain. Oxidative stress is one of the important causative factors in the pathogenesis of diabetes mellitus. Implication of free radicals was well established theory in the development of diabetes mellitus and the agents that scavenge free radicals may have great potential in ameliorating disorders like diabetes mellitus[19]. Increased oxidative stress has been postulated in the diabetic state[20]. Oxidative stress in diabetes coexists with a reduction in the anti-oxidant status[21]. In Type I diabetic rats, quercetin significantly reduced the serum glucose levels with both oral (25 mg/kg) as well as intraperitoneal (10mg/kg)administration. This implies that quercetin was found to have anti-hyperglycemic activity. This finding was in accordance with the previous study[17] and another study demonstrated that *Ginkgo biloba* containing bioflavonoids like quercetin, has shown a significant reduction in fasting blood glucose levels[22]. In type II diabetic rats, quercetin significantly reduced the serum glucose levels with both oral (25 mg/kg) as well as intraperitoneal (10mg/kg) administration. Quercetin probably acting either by the insulinomimetic activity or increasing the insulin secretion. This assumption was supported by the earlier study, grape seed derived procyanidins,a bioflavonoid produced the anti-hyperglycemic effect in streptozotocin induced diabetic rats by insulinomimetic activity in insulin sensitive cell lines. Furthermore, it has reported that quercetin has the ability to facilitate insulin secretion in diabetic rats[17]. It was suggested that the stimulatory compounds such as quercetin and (-) epicatechin may, at least in part, exert their effects on insulin release via changes in Ca^{2+} metabolism.Moreover in normal as well as diabetic (TypeI & TypeII) rats quercetin 10mg/kg ip.was produced the same extent of hypoglycemic and antihyperglycemic activity respectively, as that of effect produced by quercetin 25mg/kg orally, though intraperitoneal dose of quercetin is less than that of oral dose. In all the three groups i.e. Normal, Type I & Type II Diabetic rats, statistically significant percent glucose reduction was observed. Quercetin is exhibiting significant hypoglycemic as well as antihyperglycemic activity. Moreover antihyperglycemic activity was observed in both Type I and Type II Diabetic rats. From these results we can assume that activity might be due to enhanced insulin secretion or quercetin's insulinomimetic activity. The probable mechanism of action of quercetin is to be further established. Quercetin, a well known bioflavonoid with promising antioxidant property, it might have reduced the oxidative stress and improved the antioxidant defense status in diabetic rats. This may be the reason for more degree of observed blood glucose reduction in diabetic rats compared to normal rats. This is to be further confirmed by measuring lipid per oxidation and antioxidant enzyme levels. It was assumed that quercetin, a

bioflavonoid with antioxidant properties increases insulin release in Streptozotocin-induced diabetic rats; thus exerting its beneficial antidiabetic effects.

CONCLUSION

In Normal rats, quercetin has produced the significant peak reduction in serum glucose level at 8th hour with 25mg/kg orally as well as 10 mg/kg intraperitoneally. These results demonstrate that thequercetin has hypoglycemic activity. Similarly, in Type I &Type II diabetic rats, quercetin has produced the significant peakreduction in serum glucose level at 8thhour with 25mg/kg orally as well as 10 mg/kg intraperitoneally. It shows the anti-hyperglycemic activity of quercetin. From the results mentioned above, we can conclude that quercetin probably shows its effect either by the insulinomimetic activity or increasing the insulin secretion. Quercetin was found to have anti-hyperglycemic activity in both Type I and Type II diabetic rats. It was speculated that quercetin is probably acting by different mechanisms in Type I and Type II diabetic rats. The studies has clearly demonstrated a beneficial role of a dietary antioxidant such as quercetin on diabetic status. However, the mechanisms and probable mode of action need to be studied in detail. The effects of quercetin on antioxidant defense in streptozotocin induced diabetic rats have recently been reported[23]. Quercetin treatment may protect pancreatic cells in diabetes by decreasing oxidative stress. Bioflavonoids have received an added impulse owing to the presence of their wide variety of biological activities. Numerous studies reported the anti-hyperglycemic activity of antioxidants explains about the involvement of oxidative stress in the pathobiology of diabetes mellitus. There is scarce information with regard to the mechanism of action of quercetin as an anti-diabetic drug. However, Anti-oxidant supplements like ascorbic acid were found to have beneficial effects along with the standard anti-diabetic drugs[24]. As an anti-oxidant, quercetin has great potential to alleviate the oxidative stress. Apart from its anti-oxidant activity, it can also be a promising drug in the treatment of diabetes mellitus. This is to be established by further studies.

ACKNOWLEDGEMENT

The authors are thankful to, The AU College of Pharmaceutical sciences, Visakhapatnam, Andhra Pradesh for providing necessary facilities and support to carry out this work.

Table I: The blood glucose levels in normal as well as diabetic (Type-I & Type-II) rats after oral administration of Quercetin at the dose of 25mg/kg

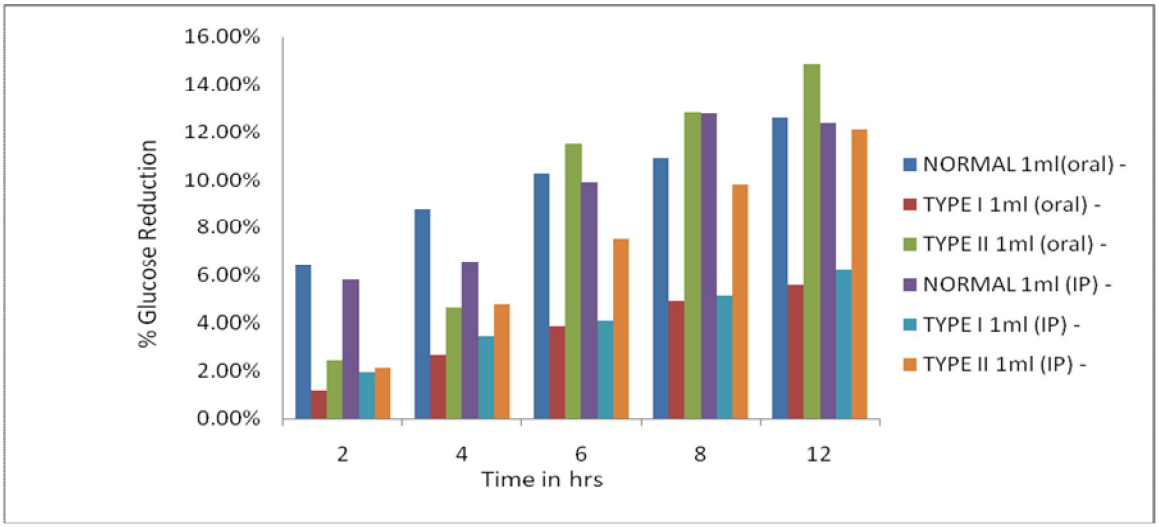
Time (hr)	Normal Rats				Type I Diabetic Rats				Type II Diabetic Rats			
	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (25mg/kg) mean ± SEM mg/dl	% Red	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (25mg/kg) mean ± SEM mg/dl	% Red	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (25mg/kg) mean ± SEM mg/dl	% Red
0	85.66±2.66	0	84±3.76	0	480±5.02	0	453.6±7.86	0	329.6±5.12	0	279.8±4,64	0
2	80.16±2.73	6.42	60.16±2.08	28.38	474.5±4.87	1.14	487.3±9.59	14.62	321.5±4.11	2.42	241.6±4.57	13.64
4	78.16±3.12	8.75	48.66±1.08	42.07	467.3±4.04	2.64	343±26.57	24.39	314.3±4.95	4.65	146.3±5.89	47.7
6	76.83±3.21	10.3	46.83±1.19	44.25	461.6±3.64	3.83	212.3±18.96	53.19	291.8±15.20	11.47	115.1±2.89	58.84
8	76.33±3.09	10.89	42.16±0.87	44.84	456.3±3.46	4.9	137.6±6.95	69.65	287.3±15.10	12.84	77.5±2.45	72.3
12	74.83±3.00	12.6	53.66±1.40	36.11	452,8±3.6	5.6	158.5±5.82	65.05	280.6±14.83	14.86	95.3±2.55	65.93

Table II: The blood glucose levels in normal as well as diabetic (Type-I & Type-II) rats after Intraperitoneal administration of Quercetin at the dose of 10mg/kg

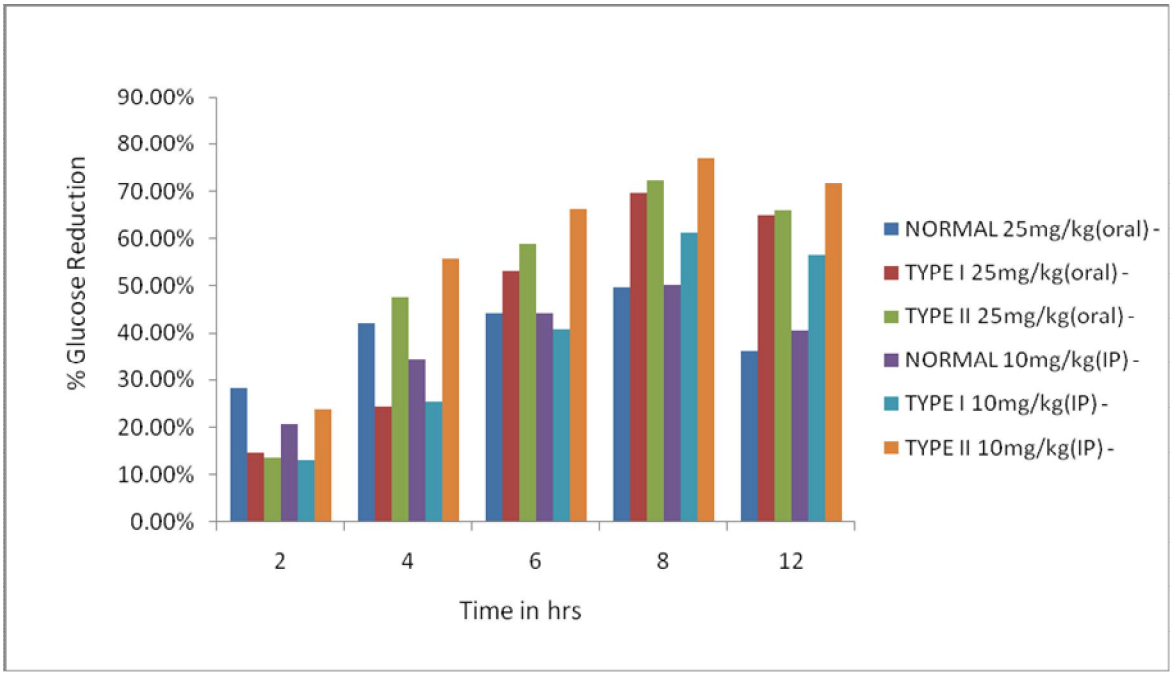
Time (hr)	Normal Rats				Type I Diabetic Rats				Type II Diabetic Rats			
	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (10mg/kg) mean ± SEM mg/dl	% Red	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (10mg/kg) mean ± SEM mg/dl	% Red	Vehicle (1%sodim CMC) mean ± SEM mg/dl	% Red	quercetin (10mg/kg) mean ± SEM mg/dl	% Red
0	86.16±3.87	0	92.66±2.26	0	444.3±5.67	0	449.1±5.24	0	330.8±4.31	0	337±14.58	0
2	81.16±3.15	5.8	73.5±1.11	20.67	435.83±6.50	1.91	390.6±5.03	13.02	323.8±4.36	2.1	257.5±10.36	23.59
4	8.5±2.48	6.56	60.83±2.15	34.35	429±6.24	3.45	334.1±6.44	25.60	314.8±4,58	4.8	149.1±3.36	55.73
6	77.66±2.99	9.86	51.5±1.05	44.42	426.3±5.88	4.05	265.3±9.06	40.92	306.0±4.61	7.5	113.6±4.04	66.27
8	75.16±3.16	12.76	46.16±1.07	50.18	421.5±5.57	5.13	174.1±10.87	61.22	298.1±4.31	9.8	77.1±4.96	77.10
12	75.5±3.88	12.37	55±1.65	40.46	416.6±5.55	6.22	195±7.32	56.58	290.5±4.66	12.1	95.6±3.87	71.61

Table III: The % blood glucose level reduction in normal as well as diabetic (Type-I & Type-II) rats

Time (hr)	% Reduction in Normal Rats				% Reduction in Type I Diabetic Rats				% Reduction in Type II Diabetic Rats			
	Vehicle (1%sod. CMC) Orally (A)	Vehicle (1%sod. CMC) IP (B)	quercetin (25mg/kg) Orally (C)	quercetin (10mg/kg) IP (D)	Vehicle (1%sod. CMC) Orally (E)	Vehicle (1%sod. CMC) IP (F)	quercetin (25mg/kg) Orally (G)	quercetin (10mg/kg) IP (H)	Vehicle (1%sod. CMC) Orally (I)	Vehicle (1%sod. CMC) IP (J)	quercetin (25mg/kg) Orally (K)	quercetin (10mg/kg) IP (L)
0	0	0	0	0	0	0	0	0	0	0	0	0
2	6.42	5.80	28.38	20.67	1.14	1.91	14.62	13.02	2.42	2.1	13.64	23.59
4	8.75	6.56	42.07	34.35	2.64	3.45	24.39	25.60	4.65	4.8	47.70	55.73
6	10.3	9.86	44.25	44.42	3.83	4.05	53.19	40.92	11.47	7.5	58.84	66.27
8	10.89	12.76	44.84	50.18	4.9	5.13	69.65	61.22	12.84	9.8	72.30	77.10
12	12.6	12.37	36.11	40.64	5.6	6.22	65.05	56.58	14.86	12.1	65.93	71.61



Graph. I: Effect of 1% sodium cmc on blood glucose level of normal and diabetic (Type I & Type II) rats



Graph. II: Effect of Quercetin on blood glucose level of normal and diabetic (Type I & Type II) rats

REFERENCES

1. Chakraborty R and Rajagopalan R. Diabetes and insulin resistance associated disorders: disease and the therapy. Current Science. 2002;83:1533-1538.
2. Kameswara Rao B, Renuka Sudarshan P, Rajasekhar MD, Nagaraju N and Appa Rao CH. Hypoglycemic activity of Terminalia pallida fruit in alloxan induced diabetic rats. J Ethnopharmacology. 2003;85:169-172.
3. Venkatesh S, Reddy GD, Reddy BM, Ramesh M and Appa Rao AVN. Antihyperglycemic activity of Caralluma attenuata. Fitoterapia. 2003;74:274-279.
4. Merken HM and Beecher GR. J Agric Food chem. 2000;48(3):577-599.
5. Harborne JB. Nature, distribution and function of plant flavonoids. In: cody, V, Middelton Elliott, Jr. and Harborne J.B.(Eds), The Flavonoids in biology and medicine: Biochemical, pharmacological and structure activity relationship, New York, USA, 1986;15-24.
6. Pinent M, Blay M, Blade MC, Salvado MJ, Arola L and Ardevol A. Grape seed Derved procyanidins have an antihyperglycemic effect in streptozotocin induced diabetic rats and insulinomimetic activity in insulin sensitive cell lines. Endocrinology. 2004;145(11):4985-4990.
7. Rui Zhao, Qingwang Li, Ling Long, Jain Li, Runjun Yang and Dawei Gao. Antidiabetic activity of flavone from Ipomoea Batatas Leaf in non-insulin dependent diabetic rats. 2007;42(1):80-85.
8. Pinakini K Shankar, Vasanth Kumar and Namita Rao. Evalution of antidiabetic activity of Ginkgo Biloba in streptozotocin induced diabetic rats. IJPT. 2005;4:16-19.
9. Saija A, Scalese M and Lanza M. Flavonoids as antioxidant agents: Important of their interaction with biomembranes. Free Radic Bio Med. 1995;19:481-486.
10. Miller AL. antioxidant flavonoids: structure function and clinical usage. Alt Med Rev. 1996;1: 103-11.
11. Chen YT, Zheng RL, Jia ZJ and Ju Y. Flavonoids as super oxide scavengers and antioxidants. Free Radic Biol Med. 1990;9:19-21.
12. De Whalley CV, Rankin JF and Rankin SM. flavonoids inhibit the oxidative modification oflow density lipo protein. Biochem Pharmacol. 1990;39:1743-1749.

13. Skaper SD, Fabris M and Ferrari V. Quercetin protects cutaneous tissue associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooparative effects of Ascorbic acid. Free Radic Biol Med. 1997;22:669-678.
14. Alarcondela, Lastra C, Martin MJ and Motilva V. Antiulcer and gastroprotective effect of Quercetin a gross and histologic study. Pharmacology. 1994;48:56-62.
15. Mizui T, Sato H, Hirose F and Doteuchi M. Effect of Antiperoxidative drugs on gastric damage induced by ethanol in rats. Life Sci. 1987;41:755-763.
16. Scambia G, Raneletti FO and Panici PB. Inhibitory effect of Quercetin on primary ovarian and endometrial cancers and synergistic activity with cis-diamminedichloroplatinum (II). Gynecol Oncology. 1992;45:13-19.
17. Mahmood Vessal M, Hemmanti M and Vasei M. Antidiabetic effect of Quercetin in streptozotocin induced diabetic rats. Comp Biochem Physiol C. 2003;135:357-364.
18. Trinder P. Ann Clin Biochem. 1964;6:24.
19. Wilson RL. Free radicals and tissue damage, mechanistic evidence from Radiation studies in Biochemical Mechanisim of Liver injury. Academic press, NewYork. 1988;123.
20. Lawrence JC, Jill SG, Eric PD, Joyle AD, Donald DC and Mark AY. Effect of antioxidant treatment on streptozotocin induced diabetic rats on endoneuiral blood flow, motor nerve conduction velocity and vascular reactivity of epineural arterioles of the sciatic nerve. Diabetes. 2001;50:1927-1937.
21. Collier A, Wilson R, Bradleyy H, Thomson JA and Small M. Free Radical activity in type II Diabetes. Diabetic Med. 1990;7:27-30.
22. Pincemail J and Deb C. In: The antiradical properties of Ginkgo biloba (Funfgeld Publications),Rokan (Egb). Recent results in pharmacology and clinic.Edited by Heidelberg, Springer Verlag. 1988;32
23. Sanders RA, Rauscher FM and Watkins JB. Effects of quercetin on antioxidant defense in streptozotocin induced diabetic rats. J Biochem Mol Toxicol. 2001;15:143–9.
24. Eriksson J. Mg and ascorbic acid supplementation in Diabetes mellitus Ann Nutr Metab. 2011;39(4):217-223.