ANTIOXIDANT AND HYPOGLYCEMIC ACTIVITY OF SOME INDIAN MEDICINAL PLANTS

Dhan Prakash[1], Pankaj Kumar[2], Neeraj Kumar[3]

[1,2,3,]Department of Pharmacy, Shri Ram Murti Smarak College of Engineering & Technology, Nainital Road, Bareilly-243202 (UP, India).

Author for correspondence Email: neerajsitm@yahoo.com

Summary

The present study was designed to evaluate comparative antioxidant and hypoglycemic activities of 10 herbal samples referred in Indian system of medicine by using alloxan induced diabetic albino rats.The 80% alcoholic extracts of *Casearia esculenta*, *Coccinia indica, Tragia involucrate*, *Moringa oleifera, Tinospora cordifolia*, *Ficus benghalensis*, *Murraya koenigii*, *Sesbania aegyptiaca*, *Mucuna prurita* and *Zingiber officinale* were separately suspended with 1% gum acacia and employed for assessing anti-diabetic activity at a dose of 200mg/kg for 21 days and glibenclamide tablet was used as a standard drug. DNA nicking assay was performed by using supercoiled pUC 18 DNA and analyzed on 1% agarose gel. *T. cordifolia* was found to be most potent and showed blood glucose lowering effect from 298 to 235 mg/dl, 186 mg/dl and 95 mg/dl after 1, 2 and 3 weeks of treatment respectively. The effect after 3 weeks in terms of hypoglycemic activity in increasing order was *M. prurita, S. aegyptiaca, M. koenigii, Z. officinale, F. benghalensis, C. esculenta, M. oleifera, T. involucrate, C. indica* and *T. cordifolia*. The total phenolic contents showed variation from 10.2 (*Zingiber officinale*) to 45.6 mg GAE/g extract (*Muraya koenigii*) and antioxidant activity from 28.9 (*Coccinia indica*) to 75.6% (*Moringa oleifera*) in the extracts of different plants. In the protection of DNA damage experiment *Moringa oleifera* and *Tinospora cordifolia* showed significant reduction in the formation of nicked DNA and increased native DNA.

Keywords: Diabetes mellitus, Alloxan, Hypoglycaemic activity; Medicinal plants, Phenols, Antioxidant activity, Free radical scavenging activity

Introduction

Diabetes mellitus (DM) is a metabolic disorder associated with increased morbidity, mortality rate and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defect in insulin secretion, insulin action or both, resulting in impaired carbohydrate, lipid and protein metabolism**.** It is a major health problem worldwide; approximately 5% of the world's population suffers from diabetes. Since DM is a multi-factorial disease, the treatment is aimed to not only controlling blood sugar level to normal limit, but also at correcting the associated metabolic defects. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidney, nerves and arteries.

Along with hyperglycemia and abnormalities in serum lipid, diabetes is associated with micro and macro vascular complications the major causes of morbidity and death due to diabetes[1-4]. Besides medicine, exercise and diet play key role in the management of diabetes mellitus. There is a great scope for exploiting the anti-diabetic potency of natural sources that appear to hold promise as potential anti-diabetic agents. More than 150 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either singly or in combinations as compound formulation[5-8]. The presence of specific Phytochemicals, inorganic micro nutrients, vitamins and antioxidants play important role to control blood sugar level and associated disorders. The minerals are not hypoglycaemic in themselves but most of the essential trace mineral elements act primarily as catalysts or co-factors in enzyme systems [9-11].
The earlier reports by different investigators showed wide variations in their experimental results for the same plant(s). That might be due to inadequate experimental design, incomplete extraction procedure, and insensitive animal model for showing great variation and some time in some cases even negative results have been reported[12-21]. The present investigations were undertaken with the objective to take care against all such factors and 10 medicinal plants known for their hypoglycaemic activities were selected. Some of these plants had been investigated earlier and some others are less known for their hypoglycaemic activity[5, 12-21].

In the present study, comparative hypoglycaemic activities of 80% ethanol/water extracts of herbal samples referred in Indian system of medicine have been thoroughly evaluated by using alloxan induced diabetic albino rats.

Materials and Methods

The specific parts of the full grown matured plants (Identified by Dr S.K. Tewari, Scientist, National Botanical Research Institute, Lucknow) were collected, cleaned thoroughly, dried and powdered. The experimental plants, part used and their family are *Casearia esculenta* Wight and Arn. (stem, Flacourtiaceae), *Coccinia indica* Wight and Arn (matured unripe fruits, Cucurbitaceae), *Tragia involucrate* L. (whole plant, Euphorbiaceae), *Moringa oleifera* Lam. (aerial parts, Moringaceae), *Tinospora cordifolia* (Willd.) Miers (stem, Menispermaceae), *Ficus benghalensis* L. (stem bark, Moraceae), *Murraya koenigii* (L) Spreng (aerial parts, Rutaceae), *Sesbania aegyptiaca* Pers. (leaves, Papilionaceae), *Mucuna prurita* Hook. (seeds, Papilonaceae) and *Zingiber officinale* Rosc. (rhizomes, Zingiberaceae). The powdered (40 Mesh) plant samples (200 g) were extracted with 80% ethanol/water (V/V, 600 ml) in a soxhlet at controlled temperature. The collected plant extracts were separately concentrated under reduced pressure below 60^0 C by using a vacuum pump and rotary evaporator ensuring complete removal of the solvent. The concentrated and dried ethanolic extracts of the samples thus obtained were stored at $4\text{-}5^0$ C until used.

Animal selection and induction of diabetes:
Wistar albino male rats weighing between 150-200 g, obtained from animal house of the college were used for the experiment. Throughout the study, animals were maintained under normal laboratory conditions and were given standard animal feed. Animal study protocol was approved by institutional animal ethical committee (IAEC). The animals were kept on fasting for 24 hrs and rendered diabetic by injecting a single dose of alloxon 150 mg/kg body weight (Loba, Mumbai) administered as a 5% w/v in distilled water.

It produced diabetes by selective necrosis of β-cells of islets of langerhans of pancreas. After one week, diabetes was confirmed by testing blood glucose by using o-toluidine method[20, 22, 23]. The animals with sugar level more than 200 mg/dl were considered as experimental diabetic. The 80% alcoholic extract of the plants were separately suspended with 1% gum acacia and employed for assessing anti-diabetic activity at a dose of 200mg/kg for 21 days and glibenclamide tablet (Aventis Pharmaceuticals, Mumbai) was used as a standard drug.

Experimental design:
A total 78 rats were divided in to 13 groups each consisting of six rats. **Group-I:** received only the vehicle (1% gum acacia) served as normal control, **Group-II:** untreated diabetic animals served as a negative control, **Group-III:** diabetic animals treated with standard drug (glibenclamide, 10mg/kg body weight) served as positive control, **Group-IV to XIII:** diabetic animals treated with 200mg/kg of 80% ethanol/water (v/v) plant extracts of different plants once a day. After an overnight fasting, each group was treated for 21 days as mentioned above. The blood samples were collected from the tail vein puncture, for the measurement of blood glucose at 0, 7, 14 and 21 days by using o-toluidine method[22, 23].

Total phenolic contents (TPC) and antioxidant activity (AOA):
The powdered plant material (1.0 g) was extracted with 50% MeOH: H_2O (2 X 20 ml), overnight at room temperature and solvent from combined extract was removed under reduced pressure. The total phenolic contents (TPC) in the extracts were measured by the method of Ragazzi and Veronese[24] and were expressed as mg gallic acid equivalent (GAE) /g extract. The antioxidant activity (AOA) of extracts was performed by auto oxidation of β-carotene and linoleic acid coupled reaction according to Emmons and Peterson[25] and was expressed as per cent inhibition relative to control. Free radical scavenging activity (FRSA) was measured by using 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) radical according to Yen and Duh[26] and the inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti radical power (ARP) were estimated and calculated as described by Kroyer[27].
Reducing capacity of extracts was determined (ASE/ml = absorbance of 1 mM ascorbic acid/ absorbance of 1 mg/ml sample) by ferric reducing - antioxidant power assay[28] using quercetin as reference standard and expressed as ascorbic acid equivalent (1mM = 1 ASE). DNA nicking assay were performed using supercoiled pUC 18 DNA by the method of Lee et al.[29] and analyzed on 1% agarose gel.

Results and Discussion

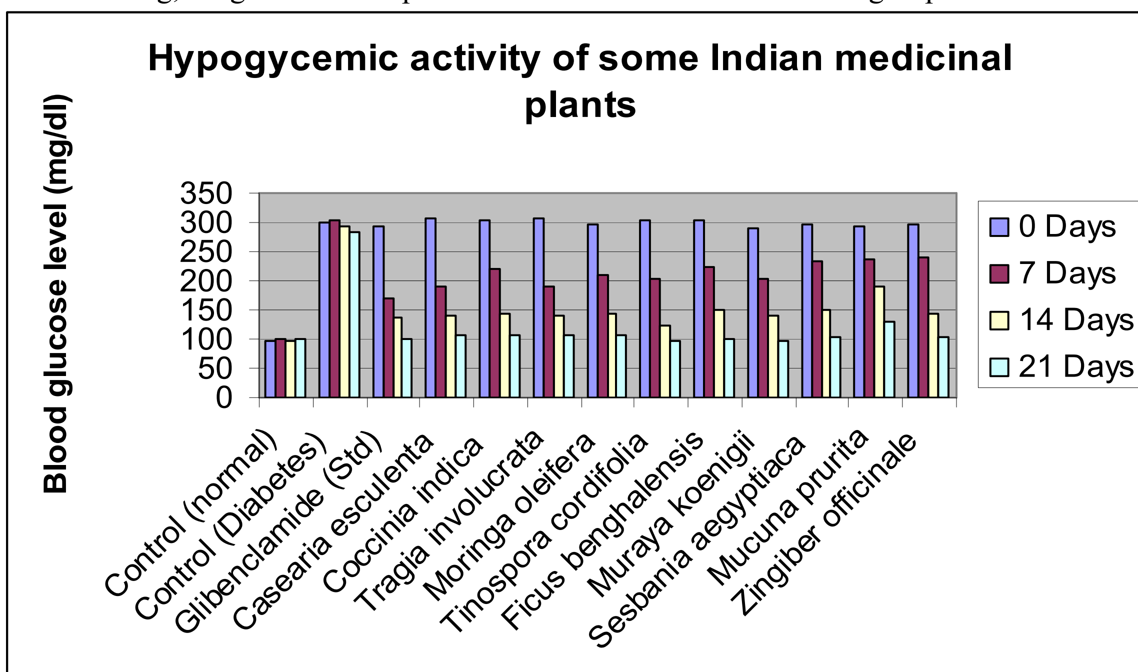
In Indian system of Ayurvedic medicine or indigenous folk medicines, the hypoglycemic plants have been mentioned to be used generally in their natural forms such as fresh juice, paste or dry powder. Dispensing natural plants in this form will retain both the inorganic and organic constituents of the concerned herbs It is also important to mention here that the inorganic part of a medicinal plant containing mainly mineral elements, sometimes plays a contributory role in enhancing medicinal properties of that particular plant or their products[9-10]. There are a number of essential minerals (Ca, Zn, K, Mn and Cr) that are known to be associated with the mechanisms of insulin release and its activity or glucose tolerance factor in different laboratory animals and also in human beings[9-11].

To study the comparative hypoglycemic effect on final blood glucose levels of each sample specific fixed doses (200mg/kg) of the concerned 80% ethanolic extracts of *C. esculenta, C. indica, T. involucrate, M. oleifera, T. cordifolia, F. benghalensis, M. koenigii, S. aegyptiaca, M. prurita* and *Z. officinale* were given to experimental animals. The blood glucose levels were measured in fasting animals at 0, 7, 14 and 21 days (Table 1). The present result indicated that most of the experimental samples showed specific blood glucose lowering effects within 2 and 3 weeks. The experimental sample of *T. cordifolia* was found to be most potent and showed blood glucose lowering effect from 298 to 235 mg/dl (21%), 186 mg/dl (38%) and 95 mg/dl (68.1%) after 1, 2 and 3 weeks of treatment respectively (Table 1).
The next higher hypoglycaemic activity, a reduction of 60.3% was observed on similar dose after 3 weeks by *Coccinia indica.* The standard drug glibenclamide used for comparison showed 63.5% decrease from initial level after 3 weeks of treatment. The marked effect of most of the samples was observed after 3 weeks and in terms of hypoglycemic activity in increasing order it was *M. prurita* (36.4), *S. aegyptiaca* (37.8), *M. koenigii* (41.7), *Z. officinale* (45.7), *F. benghalensis* (50.7), *C. esculenta* (53.9), *M. oleifera* (57.0), *T. involucrate* (58.8), *C. indica* (60.3) and *T. cordifolia* (68.1% decrease from initial level). Some of the medicinal plants are among the numerous plant adjuncts tried for the treatment of diabetes mellitus. There is scope for more extensive research in this area, especially to examine their long term beneficial effect to identify the active principle, and to understand the mechanism of action.
WHO has pointed out that prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be an attainable target. The WHO study groups in its report had emphasized strongly the need of optimum, and rational uses of traditional and natural indigenous systems of medicines in health care of general public of any specific country[1, 2]. There is still insufficient evidence to draw definite conclusions about the efficacy of individual herbs and supplements for diabetes; however, they appear to be generally safe. The available data suggest that several supplements may warrant further study. The best evidences for efficacy from adequately designed randomized controlled trials (RCTs) are available for several plants of Indian system of natural medicines[12-21].

Table 1: Effect of 80% ethanolic extracts of different plants on blood glucose level of diabetic albino rats.

Treatment	% Yield of Extract	0 Day	7 Days	14 Days	21 Days
Control (Normal)	--	96±2.3	99±3.2	97±1.4	99±10.2
Control (diabetic)	--	300±11.1	305±6.0	295±8.0	282±10.4
Glibenclamide (Std)	--	294±11.4	169±23.1	138±28.1	101±5.2*
Casearia esculenta	6.7	308±5.0	190±11.0	141±13.7	106±5.7*
Coccinia indica	8.5	303±24.4	220±17.9	142±23.3	106±8.7*
Tragia involucrata	4.3	306±9.6	191±14.8	140±16.6	107±7.7*
Moringa oleifera	9.8	296±21.9	209±16.9	145±15.4	107±7.7*
Tinospora cordifolia	10.6	304±13.0	203±13.9	122±16.0	97±2.7*
Ficus benghalensis	12.3	303±4.7	222±18.8	149±12.9	100±3.3*
Muraya koenigii	7.9	289±13.0	204±3.7	141±7.6	96±4.3
Sesbania aegyptiaca	10.2	297±7.6	232±28.7	151±15.1	104±7.4
Mucuna prurita	5.3	294±6.0	237±28.2	190±21.3	130±3.3
Zingiber officinale	11.8	298±10.6	240±20.7	143±23.3	102±2.8

Blood glucose values (in mg/dl) are the mean ± S.D. of six rats; Std=Standard anti-diabetic drug; *Significant compared with diabetic untreated control group at P< 0.05.



To find antioxidant potential, all the ten plants were studied (Table 2) for their total phenolic contents (TPC) and antioxidant activity (AOA). TPC showed wide variation from 10.2 (*Zingiber officinale*) to 45.6 mg GAE/g extract (*Muraya koenigii*), and AOA measured by auto oxidation of β-carotene and linoleic acid coupled reaction from 28.9 (*Coccinia indica*) to 75.6% (*Moringa oleifera*) in the extracts of different plants.

The amounts of TPC 23.5, 34.7, 34.8 GAE/g and AOA 41.9, 75.6 65.5% was respectively in *Tragia involucrate, Moringa oleifera* and *Sesbania aegyptiaca*. In case of *Moringa oleifera* the AOA was high in spite of low levels of TPC that might be due to presence of some other antioxidant phytochemicals. Phenols are known to be responsible for free radical scavenging activity (FRSA). The selected plants were further subjected to FRSA assayed by DPPH free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and was expressed (Table 2) in terms of IC_{50} (inhibitory concentration) that ranged from 0.11 to 0.73 mg/ml, EC_{50} (efficiency concentration) from 5.23 to 31.4 mg/mg DPPH and ARP (antiradical power) from 3.12 to 19.87. *Moringa oleifera* showed the highest FRSA followed by *Muraya koenigii* as evident by their low IC_{50}, EC_{50} and high ARP values, than rest of the plants. The reducing power expressed as ascorbic acid equivalent (ASE/ml) varied from 0.73 to 1.73 ASE/ml that indicates their potential as electron donor to scavenge free radicals.

Table 2. Total phenolic contents (TPC, mg/g seed extract) expressed as gallic acid equivalent (GAE); antioxidant activity (AOA %) measured by auto oxidation of β-carotene; Free radical scavenging activity (FRSA) measured in term of IC_{50} = inhibitory concentration (mg/ml of extract); EC_{50} = efficiency concentration (mg/mg DPPH); ARP = anti radical power and reducing power (ASE/ml) of some medicinal plants with hypoglycemic activity plants.

Plant	**TPC**	**AOA %**	**IC_{50}**	**EC_{50}**	**ARP**	**ASE/ml**
Casearia esculenta	15.3	39.4	0.73	31.40	3.12	1.22
Coccinia indica	17.3	28.9	0.62	28.16	3.54	1.43
Tragia involucrata	23.5	41.9	0.54	22.58	4.42	1.36
Moringa oleifera	34.7	75.6	0.11	5.23	19.87	1.05
Tinospora cordifolia	13.4	63.5	0.19	6.69	13.59	1.73
Ficus benghalensis	16.9	53.8	0.15	6.31	16.15	1.12
Muraya koenigii	45.6	69.7	0.13	5.64	18.59	0.73
Sesbania aegyptiaca	34.8	65.9	0.16	6.43	15.10	1.23
Mucuna prurita	11.7	47.3	0.47	20.59	4.85	1.19
Zingiber officinale	10.2	42.8	0.53	20.68	5.12	1.31
LSD at P<0.01	1.83	2.53	1.84	5.68	2.92	1.13

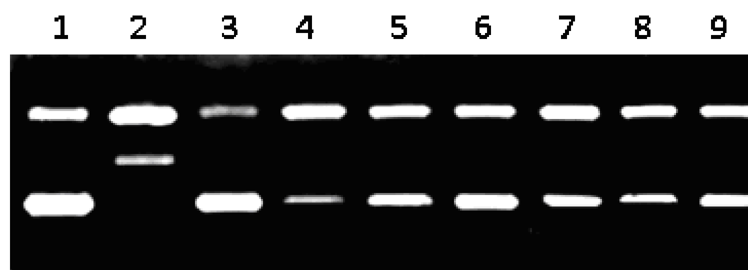


Figure 1. Inhibitory effects of some plant extracts (20 µg/ml) with hypoglycemic activity on native pUC18 DNA, nicking caused by hydroxyl radicals. **Lane 1**. pUC18 DNA; **Lane 2.** DNA + Fenton; **Lane 3.** DNA + Fenton + SOD (2U); **Lane 4**. DNA + Fenton + *Casearia esculenta;* **Lane 5**. DNA + Fenton + *Moringa oleifera;* **Lane 6**. DNA + Fenton + *Tinospora cordifolia*; **Lane 7.** DNA + Fenton + *Ficus benghalensis*; **Lane 8.** DNA + Fenton + *Tragia involucrata*; **Lane 9.** DNA + Fenton + *Cocinia indica*

In the protection of DNA damage (Fig. 1) experiment *Moringa oleifera* (Lane 5) and *Tinospora cordifolia* (Lane 6) showed significant reduction in the formation of nicked DNA (form II, circular) and increased native (form I, supercoiled) DNA. Transition metal ions are known to catalyze the formation of free radicals and reduction in the formation of single - stranded nicked DNA (form II, circular), double- stranded nicked DNA (form III, linear) and increased form I (supercoiled) DNA. Antioxidant effect of *Opuntia ficus-indica* against oxidative DNA damage had shown similar results at the same concentration[29].

The most probable reason for their potential as free radical scavengers and protection of DNA damage might be related to polyphenol and other antioxidant phytochemicals contents as they have been reported to inhibit lipid peroxidation by scavenging reactive oxygen species, chemiluminescence reactions and tumorigenesis[30, 31]. They are also known as powerful protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. Present studies together with the previous works suggest the triple synergistic action of phenols in scavenging free radicals, repairing DNA and metal chelation[29].

The phenols have well documented free radical scavenging activities and metal ion chelating capacity. They might also be responsible for their efficient free radical scavenging activity. Reactive oxygen species can cause damage to cellular bio-molecules like DNA, RNA, enzymes, lipids and carbohydrates and consequently may adversely affect immune functions. Oxidation of bases in DNA, deoxyribose lesions and strand breaks may lead to mutagenic changes and a variety of diseases[29, 32]. Phenols due to their strong antioxidant and a range of biological properties are also known to diffuse the toxic free radicals[33, 34].

From the above discussion it may be concluded that for a final co-ordinated result, the effects of both the organic and inorganic constituents of the concerned medicinal plant may be taken into consideration. Therefore, to get more potent and optimum hypoglycaemic herbs of Indian origin can be selected for their use in indigenous systems of medicine for the preparations in crude forms either singly or in combinations as compound formulations. Further besides controlling the blood sugar level the antioxidant and free radical scavenger activities may be of importance in for the protection against oxidative stress.

Acknowledgements

Authors are thankful to Mr. Dev Murti, Chairman, SRMS College of Engineering & Technology, Bareilly for his encouragement and providing research facilities and SRMS trust for financial support.

References

1. WHO, "The World Health Report. Life in the 21st Century-A Vision for All." World Health Organization, Geneva. 1998.
2. WHO, study group on diabetes mellitus. Technical report series No. 844, World Health Organisation, Geneva. 1994.
3. Hattersley A. Multiple facts of diabetes in young people. *Curr Sci* 2002; 82: 273-76.
4. Ramachandra A, Snehalata C, Vishwanathan V. Burden of Type 2 diabetes and its complications - The Indian Scenerio. Curr Sci 2002; 83: 1471-77.
5. Chaudhri RD. Herbal Drugs Industry: A practical approach to industrial pharmacognosy. Eastern Publishers New Delhi, India. 2002.
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. NISCOM, CSIR, New Delhi, India. 1999.
7. Guha Bakshi DN, Sensarma P, Pal DC. A Lexicon of Medicinal Plants in India. Naya Prakash, Calcutta, India. 1999.
8. Kapur LD. Hanbook of Ayurvedic medicinal plants. CRC Press Washington DC, USA. 2001.

9. Underwood EJ, Mertz, W. Trace elements in human and Animal Nutrition, vol. 1. Academic Press, New York, 1986. p. 11-17.
10. Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. J Ethnopharmacol 1999; 64: 179-84.
11. Tiwari A. Diabetes mellitus and multiple therapeutic approaches of phytochemical, present status and future prospects. Curr Sci 2002; 83: 30-33.
12. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol 2002; 81: 155-60.
13. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, et al. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. Diabetes 1999; 48: 2398–406.
14. Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care 2003; 26: 1277-94.
15. Grover JK, Vats V, Yadav S. Effect of feeding aqueous extract of *Pterocarpus marsupium* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. Mol Cell Biochem 2002; 241: 53-9.
16. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. Acta Diabetol Lat 1989; 26: 51-5.
17. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. J Ethnopharmacol 2002; 79: 95-100.
18. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits. J Ethnopharmacol 2003; 85: 201-16.
19. Kohli KR, Giri S, Kolhapure SA. Evaluation of the clinical efficacy and safety of Diabecon in NIDDM; The Antiseptic 2004; 101: 487-494.
20. Puri D, Prabhu KM, Murthy PS. Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. Indian J Physiol Pharmacol 2002; 46: 457-62.
21. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003; 84: 105-8.
22. Varley H, Gowenlock AH, Bell M. Practical Clinical Biochemistry, vol. 1, fifth ed. Heinemann Medical Books Ltd, London 1976. p. 389.
23. Pillai KK, Qadry JS. Biochemistry and clinical pathology 3rd ed (theory and Practical). Nirali Prakashan Delhi 2006. p 128-9.
24. Ragazzi E, Veronese G. Quantitative analysis of phenolic compounds after thin layer chromatographic separation. J Chromatography 1973; **77:** 369-75.
25. Emmons CL, Peterson DM. Antioxidant activity and phenolic contents of Oats, Groats and Hulls. Cereal Chem 1999; 76: 902 - 5.
26. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen. J Agric Food Chem 1994; 42: 629 -63.
27. Kroyer, GT. Red clover extract as antioxidant active and functional food ingredient. Innovative Food Sci Emerg Technol, 2004; 5: 101-5.
28. Apati P, Szentmihalyi K, Kristo Sz T, Papp I, Vinkler P, Szoke E, Kery A. Herbal remedies of Solidago, correlation of phytochemical characteristics and antioxidative properties. J Pharm Biomed Analysis 2003; 32: 1045 -53.

29. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. J Agric Food Chem 2002; 50: 6490-6.
30. Georgetti SR, Casagrande R, Vicenti FT MC, Verri Jr WA, Fonseca MJV. Evaluation of the antioxidant activity of soybean extract by different in vitro methods and investigation of this activity after its incorporation in topical formulations. Europ J Pharm Biopharm 2006; 17: 320-4.
31. Lee CH, Yang L, Xu JZ, Yeung SYV, Huang Y, Chen ZY. Relative antioxidant activity of soybean isoflavones and their glycosides**.** Food Chem 2005; 90: 735–41.
32. Dusinska M, Lietava J, Olmedilla B, Rašlová K, Southon S, Collins AR. Indicators of antioxidative stress in antioxidants and human health. CAB International 1999; p 411- 22.
33. Bingham M, Gibson G, Gottstein N, Pascual-Teresa SD, Minihane AM, Rimbach G. Gut metabolism and cardio protective effects of dietary isoflavones. Current Topics Nutraceutical Res 2003; 1: 31- 48.
34. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant, flavonoids and risk of coronary heart disease. The Zutphen elderly study. Lancet 1993; 342: 1007 - 11.