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Blood sugar lowering action of modified herbal formulation based on common spices

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Abstract
The present study was designed to prepare two herbal formulations, PMM1 and PMM2 using five common Indian herbs. The preclinical efficacies of these formulations were studied in alloxan induced diabetic rats. The formulations (PMM1 and PMM2) were prepared with extracts of Tinospora cordifolia, Trigonella foenum-graccum, Scoparia dulcis, Adhatoda vasica and Cassia occidental. The formulations are standardized using phytochemical analyses and HPTLC chromatographic fingerprints. Anti-hyperglycaemic activities of test drugs were evaluated on alloxan induced (120 mg/kg, i.p) diabetic rats. PMM1 (50-150 mg/kg, p.o) exhibited best potentiality in reducing blood glucose within 14 days treatment in comparison with PMM2 at the same doses levels. PMM1 treatments also significantly (p<0.5) negated elevations of blood cholesterol, triglycerides, urea and creatinine in alloxan diabetic rats. The observed effects were also comparable with Glibenclamide (5 mg/kg). The preset observation identified formulation PMM1 for anti-hyperglycemic effect and substantiates its uses on diabetic population.

Keywords: Diabetes, herbs, *Tinospora cordifolia*, starch, *Trigonella Foenum-graccum,* blood glucose

1. Introduction
Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease [1]. Recent study focused on certain diet and lifestyle modification which can contribute to islet of β-cells protection in diabetes via the modulation of cellular PI3K (Phosphatidylinositol-3kinase) pathway [2]. The use of ethno botanicals has a long folkloric history for the treatment of blood sugar abnormalities. Prior to development of insulin injection therapy in 1921 diabetes was entirely managed with herbal medicine [3]. Indian traditional medicine approach to Diabetes mellitus management includes life-style modification, dietary interventions, exercise, and a variety of hypoglycemic herbs, minerals and herbal formulas [4]. Indian traditional remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combination with immune modulators, hypocholesterolemic, antioxidants, diuretics and de toxicants [5]. The rationale behind such formulations is provided by modern research, which documents that immune processes play a predominant role in the destruction of beta cells and that free radicals feature predominantly in the progression of the disease and its secondary complications [6]. Most focused Indian traditional anti-diabetic herbs are *Tinospora cordifolia* [7], *Trigonella foenum-graccum* [8], *Melia azedarach* [9], and *Pterocarpus marsupium* [10]. Though these medicinal herbs are used in treating diabetes, but there are several reports for their adverse actions in disease condition [11-12]. Several pharmaceutical researches are undergoing to modify physicochemical properties of these herbs. There are several herbal formulations present in the markets, but still require more research to develop effective drugs to treat diabetes and its complications. In this regards, the present study was attempt to characterized the bioactive anti-hyperglycemic principles derived from five non-toxic herbs or commonly used species and herbs like, *Tinospora cordifolia* stem, *Trigonella foenum-graccum* seed, *Scoparia dulcis* areal part, *Adhatoda vasica* leaves, *Cassia occidentalis* leaf. Two separate formulation based on these five herbs were prepared and warranted their pharmacological anti-hyperglycemic activities as also diabetes related complications in alloxan induced diabetes rats.

2. Materials and Methods
2.1 Plant materials collection and authentication
Tinospora cordifolia stem, *Trigonella foenum-graccum* seed, *Scoparia dulcis* areal part, *Adhatoda vasica* leaves, *Cassia occidentalis* leaf were collected from Salipur and properly identified by Botanical Survey of India, West Bengal.

2.2 Test drug preparation

Tinospora cordifolia stem, *Trigonella foenum-graccum* seed, *Scoparia dulcis* areal part, *Adhatoda vasica* leaves, *Cassia occidentalis* leaf were used for extraction, or modification or powdered. Distilled water was used for extraction and finally lyophilized. The powdered of individual plant's were proportionately mixed in two separate formulations *viz*. PMM1 and PMM2 [13]. The two formulations are as follows:

PMM1	g%	PMM2	g%
Tinospora cordifolia	10	*Tinospora cordifolia*	10
Trigonella foenum-graccum	45	*Trigonella foenum-graccum*	15
Scoparia dulcis	20	*Scoparia dulcis*	20
Adhatoda vasica	10	*Adhatoda vasica*	10
Cassia occidentalis	15	*Cassia occidentalis*	45

2.3 Phytochemical analysis

The group analysis of individual extract and the formulated test drugs, PMM1 and PMM2 were examined for alkaloids by Dragendroff's test, sterols and triterpenoids by Liberman Buchard test, saponins by Froth test, tannins by lead acetate test, carbohydrates by Fehling's test, reducing sugars by Benedict test, proteins by Millon's test, phenolics by ferric chloride test, flavonoids by aluminum chloride test and glycosides by Borntrager's test [14].

2.4 Standardization of test formulation by HPTLC

The powdered PMM1 and PMM2 was mixed in methanol (1 mg/ml), filtered and spotted on a pre-coated silica gel plates (Merck, $60F_{254}$, 10X10 cm) using Camag Linomat 5 applicator and processed in a solvent system (toluene: ethyl acetate: formic acid=5:4:1) for 30 min. The densitometric scanning was performed on Camag TLC Scanner 3 at absorbance 280 nm (D2 lamp) operated by multi level win CATS planar chromatography manager [14]. The quercetin was used as a standard. The obtained unknown peaks were individually marked as Rf and area percent were measured. The known peak for quercetin was quantified and compared.

2.5 Animals

Swiss albino mice and Wistar rats were maintained in animal house for at least 10 days prior to experimentation. Recognized guidelines for the care and use of the animals were followed (CPCSEA guidelines for laboratory animal facility, 2003) [15]. Permission for the experiments was obtained from the institute animal ethics committee. The room temperature was maintained at 23±2 °C and humidity at 40-60%. A 12 hour light-dark cycle was also maintained. The animals were fed supplementary feed for rats (procured from Provimi, Bangalore) and water *ad libitum*. The food was withdrawn as per experimental protocol.

2.6 Acute toxicity studies

The homogenous suspension of two individual formulations were prepared freshly, using 0.5% (w/v) carboxyl methyl cellulose (CMC) using a mortar and pestle. The different groups of mice were administered various doses (0.5-2 g/kg p.o.) of extracts. The mice were then critically observed for clinical symptoms, behavioural changes and mortality up to 72 h period following OECD guidelines No.423 [16].

2.7 Selection of doses

The effective doses of blood sugar lowering action of PMM1 and PMM2 ware selected at 50, 100 and 150 mg per kg Orally [13].

2.8 Induction of experimental hyperglycaemia in rats

In overnight fasted albino rats, diabetes was induced by a single intraperitoneal injection of alloxan monohydrate in ice cold saline at a dose of 120 mg/kg body weight [17]. The diabetic state was confirmed 72 h after alloxan injection by measuring fasting blood glucose (one-touch Accu-chek sensor glucometer) from their tail vein. Rats with fasting blood glucose ≥250 mg/dl were considered to hyperglycemia (clinically resembles to diabetes) and were used in this study. The diabetic rats were divided into nine groups of six animals in each and treated orally as follows:

2.9 Treatment schedule in rats

Groups	Test Drug	Dose (orally)
1	Normal control	2 ml/kg of 0.5% (w/v) CMC
2	Diabetic control	2 ml/kg of 0.5% (w/v) CMC
3	Glibenclamide	5 mg/kg
4	PMM1	50 mg/kg
5	PMM1	100 mg/kg
6	PMM1	150 mg/kg
7	PMM2	50 mg/kg
8	PMM2	100 mg/kg
9	PMM2	150 mg/kg

All the animals were treated once daily for consecutive 14 days. The blood glucose was monitored 2 h after last dose given at 7th day and 14th day. After the experimental schedule, the rats were fasted overnight (16 h). Finally, blood was collected from heart under deep anaesthesia and serum was separated by centrifugation at 5000 rpm for 15 min and kept in –20 ºC [17]. The serum was further analyzed for biochemical estimations of total cholesterol, triglycerides, urea and creatinine using commercial kits (Span Diagnostics, India).

2.10 Statistical analysis

The data generated during the study were expressed as means ± standard error of mean. The data were analyzed statistically using software based statistical package (spss version 20, IBM, USA). The percent changes were also calculated.

3. Results

3.1 Phytochemical analysis

The results of phytochemical group analysis of all individual test compounds and formulated test drugs were Incorporate in between showed and Table 1. The formulated test compounds have all active groups, like than individual test compounds [Table 1].

Table 1: Phytochemical screening of test compounds

	Reducing sugars	Non-reducing sugars	Proteins	Tannins	Alkaloids	Triterpenoids	Glycosides	Flavonoids	Phenolics	Saponins	Sterols
Tinospora cordifolia	-	-	-	-	+	+	-	-	-	+	-
Trigonella foenum-graccum	-	+	+	-	+	-	-	+	+	+	+
Scoparia dulcis	-	-	-	-	+	+	+	+	+	+	-
Adhatoda vasica	-	+	+	+	+	-	-	-	+	+	+
Cassia occidentalis	-	-	-	+	+	+	-	-	+	+	+
Formulation: PMM1	-	+	+	+	+	+	+	+	+	+	+
Formulation: PMM2	-	+	+	+	+	+	+	+	+	+	+

+ present, - absent

3.2 HPTLC analysis

The chromatograms of PMM1 and PMM2 showed distinct features of compounds and peaks. Figure 1 represents HPTLC chromatogram of standard quercetin (Rf 0.47). PMM1 has 11 peaks [Fig. 2] and PMM2 has 9 peaks [Fig. 3]. Both of these chromatograms exhibited quercetin at Rf 0.47, but the concentrations were differed. PMM1 showed 15.7% of quercetin, whereas PMM2 exhibited only 2.4% [Table 2].

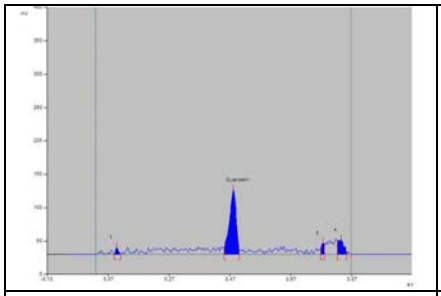

Fig 1: HPTLC of quercetin

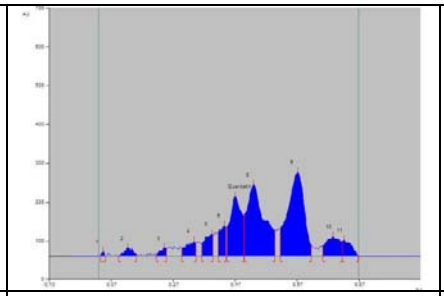

Fig 2: HPTLC of PMM1

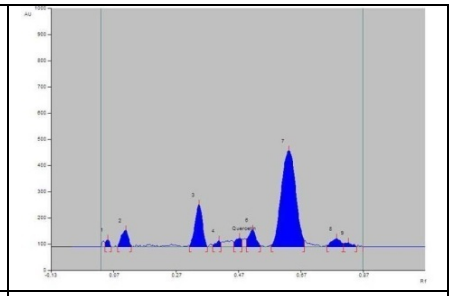

Fig 3: HPTLC of PMM2

Table 2: Compounds of PMM1 and PMM2 in HPTLC

	Rf (Area %)										
PMM1	0.03 (0.3%)	0.09 (1.6%)	0.21 (1.1%)	0.30 (3.2%)	0.36 (4.2%)	0.41 (4.5%)	0.47 (15.7%)	0.50 (28.3%)	0.61 (31.4%)	0.75 (6.2%)	0.81 (3.2%)
PMM2	0.04 (1.2%)	0.08 (5.3%)	0.31 (14.3%)	0.39 (1.3%)	0.47 (2.4%)	0.49 (5.8%)	0.57 (64.5%)	0.75 (3.5%)	0.81 (1.4%)	-	-

3.3 Acute toxicity studies

The test formulation PMM1 and PMM2 was found to be safe up to 2 g/kg body weight dose in mice.

3.4 Anti-hyperglycaemic action

Alloxan elevated blood glucose up to 358.5% within 7 days and 490.9% within 14 days compared to normal control rats. The standard oral anti-hyperglycaemic agent, glibenclamide reduced blood glucose 41% within 7 days and 58.8% within 14 days. Moreover, in combination of five ingredients in two different formulation, PMM1 showed more significant ($p<0.05$) and prominent anti-hyperglycaemic action than PMM2. PMM1 at the dose of 150 mg/kg exhibited 52.2% reduction within 14 days than PMM2 that lowered only 48.4% at the same dose and time interval [Table 3].

Table 3: Anti-hyperglycaemic potency of test formulations on alloxan induced Wistar rats

Group	Test Drug	Dose	Blood glucose		
			Day 0	Day 7	Day 14
1	Normal Control	2 ml/kg 0.5% CMC	68.1±5.34	69.3±6.08	68.8±7.13
2	Diabetes Control (Alloxan)	2 ml/kg0.5% CMC	67.3±5.35(a)	317.8±5.34(a)*	406.6±12.30(a)*
3	Glibenclamide	5 mg/kg	69.3±7.39(b)	187.5±8.09(b)*	167.3±9.01(b)*
4	Diab + PMM1	50 mg/kg	69.1±4.95(b)	267.1±7.25(b)*	232.5±12.53(b)*
5	Diab + PMM1	100 mg/kg	69.0±6.69(b)	217.6±7.39(b)*	194.3±5.81(b)*
6	Diab + PMM1	150 mg/kg	69.3±7.99(b)	197.1±7.35(b)*	173.0±11.74(b)*
7	Diab + PMM2	50 mg/kg	68.8±3.18(b)	290.5±8.01(b)	252.8±9.30(b)*
8	Diab + PMM2	100 mg/kg	68.1±3.86(b)	242.0±7.58(b)*	223.1±8.93(b)*
9	Diab + PMM2	150 mg/kg	69.5±7.14(b)	227.1±8.54(b)*	209.5±6.09(b)*

Data are Mean ± SD (N=6); (a) means compared to normal control, (b) means compared to diabetic control on same day; Diab means alloxan induced diabetic control; * mean $p<0.05$

3.5 Lipid lowering action

Alloxan diabetic rats elevated serum cholesterol (158.6%) and triglycerides (181.9%) than normal control rats. Glibenclamide exhibited 28.8% reduction of cholesterol and 36.6% triglycerides within 14 days. PMM1 and PMM2 significantly ($p<0.05$) and dose dependently reduced the lipid profile. PMM1 at the dose of 150 mg/kg lowered 34.3% cholesterol than control diabetes rats. But, PMM2 exhibited only 28% reduction at the same dose. PMM1 at the dose of 150 mg/kg lowered 51.6%, while, PMM2 reduced only 41.9% [Table 4].

Table 4: Anti-lipidemic potency of test formulations on alloxan induced Wistar rats

Group	Test Drug	Dose	Total Cholesterol	Triglycerides
1	Normal Control	2 ml/kg 0.5% CMC	69.6±7.44	97.1±5.26
2	Diabetes Control (Alloxan)	2 ml/kg0.5% CMC	180.0±10.19(a)*	273.8±9.70(a)*
3	Glibenclamide	5 mg/kg	128.1± 10.16(b)*	173.5± 14.33(b)*
4	Diab + PMM1	50 mg/kg	162.5±11.00(b)*	179.5± 9.66(b)*
5	Diab + PMM1	100 mg/kg	145.8±9.84(b)*	157.8± 11.12(b)*
6	Diab + PMM1	150 mg/kg	118.1±6.01(b)*	132.3± 12.43(b)*
7	Diab + PMM2	50 mg/kg	164.8±14.89(b)*	192.5±11.44(b)*
8	Diab + PMM2	100 mg/kg	146.1±14.07(b)*	171.3±8.23(b)*
9	Diab + PMM2	150 mg/kg	129.6±8.04(b)*	159.0± 18.43(b)*

Data are Mean ± SD (N=6); (a) means compared to normal control, (b) means compared to diabetic control on same day; Diab means alloxan induced diabetic control; * mean $p<0.05$

3.6 Renoprotective action

Blood urea and creatinine concentration were significantly ($p<0.05$) increased in Alloxan induced diabetic rats. Two formulations, PMM1 and PMM2 showed significant in lowering blood urea and creatinine level, though PMM1 showed most promising renoprotective activity in alloxan induced diabetic condition [Table 5].

Table 5: Renoprotective potency of test formulations on alloxan induced Wistar rats

Group	Test Drug	Dose	Urea	Creatinine
1	Normal Control	2 ml/kg 0.5% CMC	16.6±1.21	0.45±0.02
2	Diabetes Control (Alloxan)	2 ml/kg0.5% CMC	34.8±2.56(a)*	1.17±0.01(a)
3	Glibenclamide	5 mg/kg	19.6± 1.96(b)*	0.77± 0.12(b)*
4	Diab + PMM1	50 mg/kg	21.6± 2.06(b)*	1.01±0.06(b)*
5	Diab + PMM1	100 mg/kg	20.6±2.16(b)*	0.84±0.10(b)*
6	Diab + PMM1	150 mg/kg	19.3±2.06(b)*	0.68±0.026(b)*
7	Diab + PMM2	50 mg/kg	22.8± 2.04(b)*	1.04±0.06(b)*
8	Diab + PMM2	100 mg/kg	22.0± 1.89(b)*	0.98±0.06(b)*
9	Diab + PMM2	150 mg/kg	21.3± 3.55(b)*	0.79±0.06(b)*

Data are Mean ± SD (N=6); (a) means compared to normal control, (b) means compared to diabetic control on same day; Diab means alloxan induced diabetic control; * mean $p<0.05$

4. Discussion

The cytotoxic action of alloxan (known diabetogen for animals) in pancreatic β-cells is mediated through reactive oxygen species (ROS) [18]. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells [19]. *Tinospora cordifolia* is widely used in Indian Ayurvedic medicine for treating diabetes. The plant is mainly used to improve the immune system and the body resistance against infections [7]. Similarly, *Trigonella foenum-graecum* is extensively used in diabetes and its related complication [8]. *Scoparia dulcis* is traditionally used as blood purifier and also employed in cardiac and urinary disorders. Extracts of this plant have been reported for anti-hyperglycaemic and antioxidant action [20]. *Adhatoda vasica* is most well-known for its effectiveness in treating respiratory conditions. It has hepatoprotective, anti-bacterial, gastro-protective actions [21]. *Cassia occidentalis* leaf has been used as a folk medicine for laxative and purgative and liver disorders [22]. Previously, it has been reported that *T. cordifolia* and *T. foenum-graecum* have antioxidant and hypoglycaemic action on alloxan induced diabetic rats [7-8]. In this study, alloxan induced rats showed prominent hyperglycaemia. PMM1 and PMM2 are formulated with the same five ingredients but only differed in mixing ratios. The differences in compositions were also reflected in HPTLC chromatograms. PMM1 established 11 distinct peaks for different compounds, while PMM2 has only nine peaks. However, quercetin is more abundant in PMM1 (15.7%) than PMM2 (2.4%). These evident basically comply that though the formulations are derived from same components but they differed chemically; and these differences clearly reflected on their biological potentialities. Even though these two formulations have anti-hyperglycaemic properties, but PMM1 has better efficacy than the other one. It may be either due to presence of purified *T. cordifolia* in higher ratio or may be due to privileged of quercetin. The bioactive water soluble anti-hyperglycaemic principle of *T. cordifolia* has been identified and known as amylopectin. This principle has been shown to enhance insulin secretion and improve glucose metabolism, thereby lowering blood sugar [23]. Interestingly, PMM1 has more or less similar efficacy in comparison to oral sulfonylurea, glibenclamide. Shorr *et al* (1996) reported the risk of hypoglycaemia in the elderly after using glibenclamide [24].

Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolysis triglycerides, but in uncontrolled diabetes, elevation in blood lipids concentration attributes the risk in coronary artery disease [25]. The abnormal high concentration of serum lipids in the diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat droplets, since insulin inhibits the hormone sensitive lipase [26]. Experimentally, alloxan induced diabetic hyperglycaemia is accompanied by increase in serum cholesterol and triglycerides levels [27]. Moreover, oral

hypoglycaemic agents are normally metabolized or cleared by the kidneys and so accumulate in uraemic patients thus increasing the risk of renal malfunction. In this study, treatment with PMM1 and PMM2 showed a significant decrease in total cholesterol, triglycerides, urea and creatinine level in diabetic rats. Perhaps, PMM1 has exhibited maximum anti-hyperglycaemic and anti-lipidemic action and proved to be more effective on alloxan-induced renal dysfunctions. It is very difficult to mention which of the active constituents was responsible for these favourable responses. According to Ayurvedic texts, a combination of substances is used to get the enhanced desired action and eliminate unwanted side effects. These active constituents may aid absorption of active principles responsible for hypoglycaemic action and also protective action on kidneys.

5. Conclusions

On the basis of results obtained, it can be conclude that PMM1 is the most effective blood glucose reducing, lipid lowering and renoprotective action and may be helpful in the therapeutic management of diabetes in near future.

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7. References

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus. Australasian Med J. 2014; 7:45-48.
2. Kitagishi Y. Certain diet and lifestyle may contribute to islet β-cells protection in type-2 diabetes v*ia* the modulation of cellular PI3K/AKT pathway. The Open Biochem J. 2014; 8:74-82.
3. Clifford IB, Caroline D. Traditional plant medicines as treatments for diabetes. Diabetes Care. 1989; 12:553-564.
4. Weaver LJ, Narayan KM. Reconsidering the history of type 2 diabetes in India: Emerging or re‐emerging disease? Natl Med J India. 2008; 21:288-91.
5. Satyavati GV, Gupta AK, Tandon N. Medicinal Plants of India. Vol. II. Indian Council of Medical Research, New Delhi, 1987.
6. Mishra LC. Scientific basis of Ayurvedic therapies. CRC Press, New York; 2004, 101.
7. Prince PS, Menon VP. Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. J Ethnopharmacol. 1999; 65:277-81.
8. Sharma RD. Effect of fenugreek seeds and leaves on blood glucose and serum insulin responses. Nutrition Res. 1986; 6:1353-64.
9. Chakraborty BK. Pancreatic beta-cell regeneration: a novel anti-diabetic mechanism of *Pterocarpus marsupium* Roxb. Ind J Pharmacol. 1980; 12:123-27.
10. Chakraborty BK. Pancreatic beta-cell regeneration: a novel anti-diabetic mechanism of *Pterocarpus marsupium* Roxb. Ind J Pharmacol. 1980; 12:123-27.
11. Martins E. The growing of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014; 4:1-10.
12. Chen W, Zhang Y, Yang G, Liu JP. Chinese herbal medicine for diabetic peripheral neuropathy. Cochrane Database Syst Rev. 2013; 10:CD007796
13. Sinha Mahapatra PK, Mandal SC. Antidiabetic activity study of neem gulancha starch and preparation of artificial neem gulancha starch without destroying neem gulancha plant. International Seminar on Promotion, Prevention and Pacification Ayurvedic Landscape, Kolkata, 2016, 140-141.
14. Sur TK, Hazra A, Hazra AK, Bhattacharyya D. Antioxidant and hepatoprotective properties of Indian Sunderban mangrove *Bruguiera gymnorrhiza* L. leave. J Basic Clinical Pharmacy. 2016; 7:75-79.
15. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol. 2003; 35:257.
16. OECD guideline for testing of chemicals. 423. Acute Oral Toxicity – Acute Toxic Class Method. Adopted: 17th, 2001.
17. Sur TK, Seal T, Pandit S, Bhattacharyya D. Hypoglycemic properties of a mangrove plant, *Rhizophora apiculata* Blume. Natural Product Sci. 2004; 10:11-5.
18. El-Missyry MA, Othman AI, Amer MA. L-Arginine ameliorates oxidative stress in alloxan induced experimental diabetes mellitus. J Appl Toxicol. 2004; 24:93-7.
19. Szkudelski T. The mechanism of alloxan and streptozotocin action on beta cells of the rat pancrease. Physiol Res. 2001; 50:536-46.
20. Muniappan L, Pari LM, Bhonde SS. Scoparia dulcis, a traditional antidiabetic plant protects against streptozotocin induced oxidative stress and apoptosis *in vitro* and *in vivo*. J Biochem Mol Toxicol. 2004; 18:261-272.
21. Gangwar AK, Ghosh AK. Medicinal uses and Pharmacological activity of *Adhatoda vasica*. Int J Herbal Med. 2014; 2:88-91.
22. Vijayalaksmi S, Ranjitha J, Devi RV, Bhagiyalakshmi M. Pharmacological profile of *Cassia occidentalis*: A Review. Int J Pharm Pharm Sci. 2013; 5:29-33.
23. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian J Pharmacol. 2003; 35:83-91.
24. Shorr RI, Ray WA, Daugherty JR, Griffin MR. Individual sulfonylureas and serious hypoglycemia in older people. J Am Geriatr Soc. 1996; 44:751-755.
25. Arvind K, Pradeep R, Deepa R, Mohan V. Diabetes and coronary artery disease. Indian J Med Res. 2002; 116:163-76.
26. Pari L, Latha M. Anti-diabetic effect of *Scoparia dulcis*: Effects on lipid peroxidation on streptozotocin diabetes. Gen Physiol Biophys. 2005; 24:13-26.
27. Rahman MM, Mostofa M, Chowdhury EH. Anti-diabetic effect of neem and spirulina in alloxan induced diabetic mice. Int J Curr Res Acad Rev. 2014; 2:124-34.