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Studies on Methanol Extract of *Momordica charantia* Against Hyperglycemic Effect in Alloxan Induced Diabetic Male *Albino* Rats –A Phytochemical Approach by GC-MS Method

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and is the most prevalent non communicable disease in the world. Blood glucose level is increased due to derangement in carbohydrate, fat, and protein metabolism. Diabetes mellitus is associated with absolute or relative deficiencies in insulin secretion, insulin action or both. The aim of the study was to investigate the anti hyperglycemic effect of methanol extract of *Momordica charantia* on alloxan induced diabetes male *albino* rats. The plant extract was orally fed to the experimental rats at dosage of 250 and 350 mg/kg of body weight. Effect of the methanol fruit extract of *Momordica charantia* on body weight, serum insulin, blood glucose, and lipid profile in normal, diabetic control and experimental rats were observed. Administration of plant extract to the experimental rats revealed there was a progressive increase in body weight and insulin level and also fall in blood sugar level. The level of lipid profile reverted to normal level in experimental rats. These results were on par with the standard drug glibenclamide. A phytochemical analysis of methanol fruit extract of *Momordica charantia* was done by GC-MS method. The identified phytochemicals may help to develop a drug for the treatment of diabetes mellitus.

Keywords: Hyperglycemia, *Momordica charantia* , Alloxan, Methanol, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic and major endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a

growing health problem in most countries and its incidence is considered to be high all over the World.[1] It is also associated with long-term complications, including retinopathy, nephropathy, neuropathy, angiopathy and several others.[2] According to World Health Organization projection, the diabetes population is likely to increase to 300 million or more by the year 2025. The current studies in India indicate that there is an alarming rise in prevalence of diabetes which has gone beyond epidemic form to a pandemic one. Globally, diabetes mellitus presents enormous and increasingly important public health issues.[3]

There are several drugs in clinical practice for the treatment of diabetes mellitus. Many of these oral antidiabetic agents have been reported to show serious adverse effect such as liver problems, lactic acidosis and diarrhea. In addition, they are not suitable for use during pregnancy. It is apparent that due to the side effects of the currently used drugs, there is a need for a potent drug with minimal adverse effects, which can be taken for long durations.[4]

Currently enormous research interest is centered worldwide about the search for newer, cheaper, and safer herbal based formulations which can effectively normalize the metabolic derangement underlying the onset of clinical diabetes.[5] Herbal medications have been used for the treatment of variety of ailments, a huge number of population in the world is entirely dependent on traditional medicines. A number of medicinal plants and their formulations are used for treating diabetes in ayurvedic medicine system as well as in ethno medicinal practices.[3] *Momordica charantia*, which is known as bitter gourd or bittermelon is a common edible vegetable and also as folk medicine especially for diabetes in Asia.

The present study was carried out to test the efficacy of methanol fruit extract of *Momordica charantia* against hyperglycemic in alloxan induced diabetes male *albino* rats. A phytochemical analysis of methanol fruit extract of *Momordica charantia* was done by GC-MS method.

MATERIALS AND METHODS

Plant material

The unripe fruits of *Momordica charantia* were collected in and around Vellore District, Tamilnadu, India. The fruits were cleaned with distilled water and shade dried at room temperature.

Preparation of plant extract

Fresh unripe fruits of *Momordica charantia* were collected, washed and cut into small pieces. The fruits were dried in shade and powdered. About 100 gms of dried powdered fruits of *Momordica charantia* were taken and mixed with 500 ml of methanol and magnetically stirred in a separate container overnight at room

temperature. The residue was removed by filtration and the extracts were concentrated under vacuum to get solid yield of 7%. The plant extract was administered orally to animals in aqueous solution.[6]

Animals

Male *albino* Wister rats weighing around 180-200 gms were purchased from Tamilnadu Veterinary and Animal Science University, Chennai, India. The animals were kept in polypropylene cages and maintained in an animal room, under controlled temperature of 25±2ºC. Humidity and airflow conditions with a 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow. They had free access to water. The experimental protocol was conducted in accordance with the institutional guideline.[6]

Experimental induction of diabetes

Diabetes was induced in the rats by the administration of single intraperitoneal injection of alloxan monohydrate (150mg/kg of body wt) (SD Fine Chem. Limited, Mumbai) in normal saline.[7] After two days the alloxan induced rats were screened for diabetes. All animals were allowed free access to water and pellet diet and maintained at room temperature in polypropylene cages.

Experimental design

Group I: Normal rats.

Group II: Diabetic induced control rats (Alloxan induced).

Group III: Diabetic induced animals fed with methanol fruit extract of *Momordica charantia* (250 mg/kg of body weight) for 30 days.

Group IV: Diabetic induced animals fed with methanol fruit extract of *Momordica charantia* (350 mg/kg of body weight) for 30 days.

Group V : Diabetic rats treated with stranded drug glibenclamide (600 µg/kg of body weight).[8]

Sacrifice study

At the end of the experimental period, the animals were deprived of food overnight, anaesthetized and then sacrificed by decapitation. Blood was taken from the jugular vein and collected in tubes. Centrifuge the blood and collect the serum for biochemical analysis. In each group, six animals were maintained for 30 days, the body weight and blood glucose was measured daily.

Body weight

The weight of the normal, diabetic control, experimental animals and drug treated animals were studied. The weight of the individual animals was measured gravimetrically from the first day to the end of treatment.

Biochemical analysis

Blood glucose level: The blood was collected from the tip of the tail vein from the rats and the blood glucose was measured using Gluco Chek glucose estimation kit (Aspen diagnostic (P) Ltd. Dehil, India).

Estimation of plasma Insulin levels

Plasma insulin was estimated using Radio Immuno Assay (RIA) kit supplied by Linco research Inc, Stat diagnostic, Mumbai, India.

Estimation of lipid profile in blood samples

On completion of the treatment, blood samples were collected and lipid profiles for all groups of animals were measured using commercially available kits. Total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) cholesterol levels in serum were determined according to the instruction of the manufacturer (Transasia Bio Medical Limited, Mumbai, India). For the determination of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol Friedewald's formula which states: VLDL cholesterol = Triglycerides/5 and LDL cholesterol = Total cholesterol – (VLDL + HDL cholesterol) was used.[9]

Phytochemical Analysis

Phytochemical analysis was done by Gas Chromatography-Mass Spectroscopy (GC-MS) method. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.[10]

Statistical analysis

The results were expressed in mean ± standard deviation (SD). Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

RESULT

Effect of *Momordica charantia* methanol extract on body weight, blood glucose and serum insulin levels of normal, diabetic control, experimental rats and drug treated rats

Table -1 demonstrates the body weight, blood glucose and serum insulin levels in normal, diabetic control, experimental rats and drug treated animals. In diabetic control animals, the body weight was significantly decreased by 22.80 %, when compared to the levels in normal animals. In *Momordica charantia*

methanol extract treated groups, the body weight increased significantly by 9.73 % in 250 mg/kg of body weight and 15.37 % in 350 mg/kg of body weight, when compared to diabetic control groups.

In alloxan induced diabetic control animals, the level of blood glucose elevated significantly by 278.05 % compared with normal groups. After administration of *Momordica charantia* methanol extract to Group III and Group IV animals (250mg/kg of body weight and 350mg/kg of body weight) the level of glucose decreased by 45.10 % and 64.11 % respectively, whereas in glibenclamide treated animals the glucose level decreased by 64.20 %, compared to the diabetic control animals.

The level of the serum insulin in Group II diabetic control animals was decreased by 46.10 %, when compared to Group I animals. In Group III and IV animals, the insulin levels showed a significant ($P < 0.001$) increase by 44.37 % and 73.79 % respectively; when compared to the diabetic control animals. This increase was nearly equal to the level of insulin in glibenclamide administrated Group V animals, which was 76.45 % when compared to diabetic control Group II animals.

Effect of *Momordica charantia* methanol extract on lipid profile of normal, diabetic control, experimental rats and drug treated rats

Table -1 represented the levels of lipid profile such as TC, TG, LDL, VLDL and HDL in normal, diabetic control, plant extract treated rats and drug treated rats. In diabetic control Group II animals the levels of TC, TG, LDL and VLDL were elevated, when compared to the normal Group I animals. After administration of *Momordica charantia* methanol extract to Group III and Group IV animals there was depletion in TC by 22.62 % and 40.42 %, TG by 42.13 % and 50.88 %, LDL by 30.46 % and 59.56 % and VLDL by 42.11 % and 50.87 % respectively. Also, there was a significant increase in HDL by 100 % in 250 mg/kg of body weight and 148.95 % in 350 mg/kg of body weight as compared to Group II diabetic animals. As expected in glibenclamide treated Group V animals, the increased levels of TC, TG, LDL, VLDL and decreased HDL reverted to near normal values. The levels of TC, TG, LDL, VLDL and HDL in normal, diabetic control, plant extract treated rats and drug treated animals were also represented in Figure -1.

Analysis of result from the Table -1 shows that there was a progressive increase in body weight and insulin level and also fall in blood sugar level after the intake of *Momordica charantia* methanol extract. The level of lipid profile reverted to normal level in experimental rats. These results were on par with the standard drug.

The phytochemical analysis of *Momordica charantia* in methanol extracts by GC-MS method

A complex mixture of many constituents was identified in methanol extract of *Momordica charantia* by GC- MS method. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the methanol extract of *Momordica charantia* are presented in Table-2. The chromatogram showed seven peaks in this plant component (Figure – 2). The identified Phychemicals are given in Table-2.

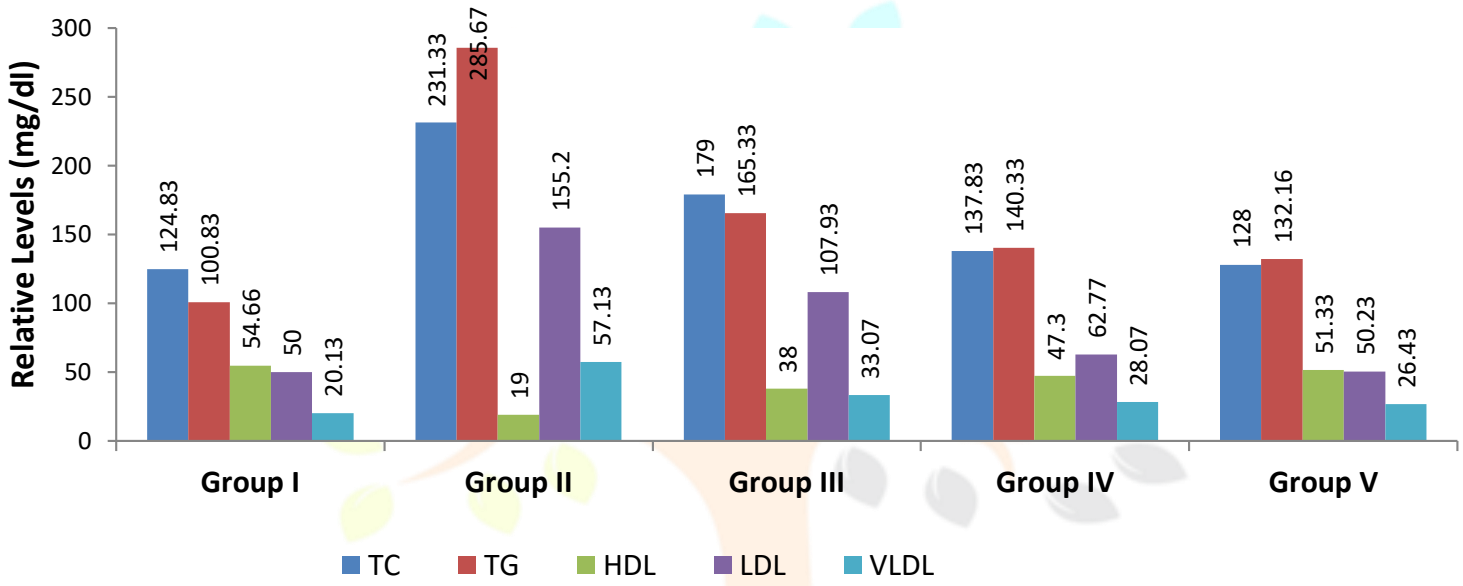
Table 1: Effect of the methanol extract of *Momordica charantia* on body weight (gms), serum insulin (µu/ml), blood glucose (mg/dl), and lipid profile (mg/dl) in normal, diabetic control, experimental rats and drug treated rats.

Parameters	(Group I) Normal rats	(Group II) Diabetic control rats	% of changes (Group I vs Group II)	Plant extract treated groups (Experimental rats)				(Group V) Drug treated	% 0f changes (Group II vs Group V)
				(Group III) 250mg/kg body wt	% of changes (Group II vs Group III)	(Group IV) 350mg/kg body wt	% of changes (Group II vs Group IV)		
Body weight	195±4.87	150.67±2.81	-22.80	165.33±2.83	9.73	173.83±3.41	15.37	182.3±3333.93	21.01
Serum insulin	57.83±1.36	31.17±0.76	-46.10	45.00±1.74	44.37	54.17±0.88	73.79	55.00±1.41	76.45
Blood glucose	99.50±1.57	376.16±4.40	278.05	206.50±2.91	-45.10	135.00±3.35	-64.11	134.67±4.41	-64.20
Lipid profile									
TC	124.83±1.64	231.33±1.81	85.32	179.00±3.00	-22.62	137.83±2.37	-40.42	128.00±5.02	-44.66
TG	100.83±1.81	285.6±3.22	183.32	165.33±2.86	-42.13	140.33±2.98	-50.88	132.16±3.06	-53.74
HDL	54.66±1.25	19.00±1.52	-65.24	38.00±1.78	100	47.30±1.56	148.95	51.33±2.80	170.16
LDL	50.00±2.03	155.20±2.36	210.4	107.93±3.92	-30.46	62.77±2.37	-59.56	50.23±2.23	-67.64
VLDL	20.13±1.23	57.13±1.3	183.81	33.07±2.25	-42.11	28.07±2.39	-50.87	26.43±0.61	-53.74

The data were expressed as mean ± SD and each value represents six individual observations, evaluated one-way ANOVA 'P' denotes the statistical significance, P<0.001. Diabetic control was compared with normal and treated groups were compared with diabetic control animals. + & - indicated the percentage of change over the diabetic control and treated groups.

Figure 1

The effect of *Momordica charantia* in methanol extract on lipid profile of normal, diabetic control, experimental rats and drug treated rats.



The data were expressed as mean ± SD and each value represents six individual observations, evaluated one-way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, P<0.001. Diabetic control was compared with normal control and treated groups were compared with diabetic control.

Group I – Normal, **Group II** – Diabetic Control, **Group III** – *Momordica charantia* Treated (250 mg/kg bw), **Group IV** - *Momordica charantia* Treated (350 mg/kg bw), and **Group V** – Drug Treated.

Figure-2: The phytochmical analysis of *Momordica charantia* in methonal extracts by GC-MS method

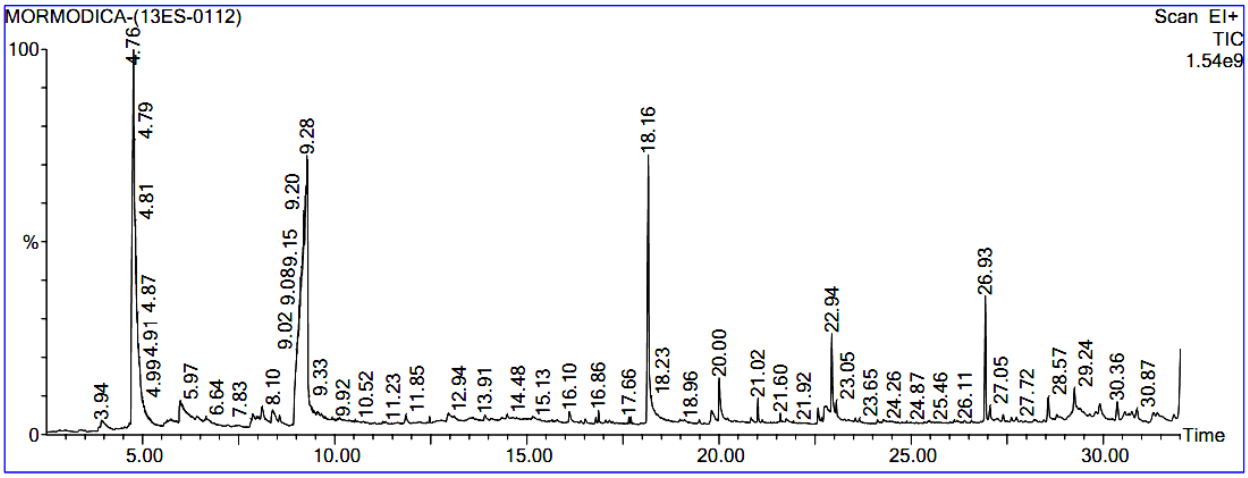


Table-2: Components detected from Methanol extract of *Momordica charantia* by GC-MS method

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	4.769	2-Methylthiolane,s,s-dioxide	C5H10O2S	134	35.167
2	9.28	Beta-1,5-o-dibenzoyl-ribofuranose	C19H18O7	358	16.461
3	18.16	N-Hexadecanoic acid	C16H32O2	256	10.822
4	5.98	Cyclohexanone,4-methyl	C7H12O	112	3.416
5	20.00	Octadecanoic acid	C18H36O2	284	1.926
6	22.94	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethly)ethly ester	C19H38O4	330	2.438
7	26.92	Cholesta-4,6-dien-3-ol,(3.beta.)-	C27H44O	384	10.48

DISCUSSION

Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug.[11] Many Indian plants have been investigated for their beneficial use in diabetes and its complications. During the study, it was observed that *Momordica charantia* have potent antidiabetic activity.

In diabetic control animals, the level of blood glucose was increased and the body weight and serum insulin level was decreased. The loss of body weight is caused by the loss or degradation of structural proteins[12]. The weight loss was reversed after treatment with the methanol extract of *Momordica charantia*.

Diabetes mellitus showed a reduction in body weight, reciprocal in direction to blood glucose, clearly indicating that the deterioration in the glucose control mechanism progresses in stages and would probably climax in the death of the animal if left untreated.[13] After administration of plant extracts the blood glucose level was reduced and serum insulin level was increased to near normal level without causing hypoglycemic state.

The administration of alloxan to the animal selectively destroys the insulin producing β-cells in the pancreas. This damage may be the reason for the lesser amount of insulin secretion. Similar results were observed in the present study. Alloxan induced diabetic rats were treated with methanol extract of *Momordica charantia* at the dose of 250 mg/kg of body weight and 350 mg/kg of body weight showed fall in blood glucose and increase in body weight and serum insulin. This finding is supported by Puri *et al.,* 2002[14] who isolated an active compound from fenugreek and showed its hypoglycemic properties in diabetic rabbits. The authors found significant attenuation of the glucose tolerance curve and improvement in the glucose induced insulin response, suggesting that the hypoglycemic effect may be mediated through stimulating insulin producing beta-cells of the Islets of Langerhans.

Plasma lipid level is usually raised during diabetes and presents a risk factor for coronary heart disease. Lowering plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease. An increase in serum total lipids, triglycerides and total cholesterol levels were observed in diabetic patient.[15, 16] The changes were also observed in diabetic induced rats. After administration of methanol extracts of *Momordica charantia* the levels of TC, TG, LDL and VLDL were decreased and HDL level was increased.These results were on par with the standard drug glibenclamide.

From the analysis of the results, it was evident that there was a progressive fall in blood sugar level and significant increase in body weight, and serum insulin. Similar significant changes were also observed in lipid profile after the intake of methanol extracts of *Momordica charantia*. These results were on par with the standard drug glibenclamide. In this study the identified phytochemical of *Momordica charantia* in methanol extract helps in future to innovate a new drug for the treatment of diabetes mellitus.

CONCLUSION

The present study reveals the antidiabetic activity of methanol extracts of *Momordica charantia* produce a marked decrease in blood glucose levels in experimental rats. The findings suggest that the methanol extract of *Momordica charantia* at the dosage of 350 mg/kg of body weight had a major potent hypoglycemic property. The identified phytochemicals may help in future research to develop a drug for the treatment of diabetes mellitus.

REFERENCES

1. Tharkar S, Devarajan A, Kumpatla S, Viswanathan V. The socioeconomics of diabetes from a developing country: a population based cost of illness study. *Diabetes Res ClinPract*, 89: 334-340, (2010)

2. Kristova V, Liskoya S, Sotnikova S, Vojtko R, Kurtansky A. Sulodexide improves endothelial dysfunction in streptozotocin-induced diabetes in rats. *Physiol Res*, 57: 491-494, (2008)
3. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti hyperglycemic potential of Tridaxprocumbens (Linn.).*BMC Complement Altern Med*, 9: 48, (2009)
4. Rajalaksmi M, Eliza J, Cecilia E, Nirmala A, Daisy P. Antidiabetic properties of Tinosporacordifolia stem extracts on streptozotocin induced diabetic rats. *Afr J Pharm Pharmacol*, 3: 171-180, (2009)
5. Paulose KP, Regi Jose, Augusti KT, Joseph PK. Diabetes mellitus and its management edited by Dr.Paul Augustine, Regional cancer centre, Thiruvananthapuram and published by Health Forum of the School of medical education, M.G.University, Kottayam, PP 6 – 46: 65-84, (2001)
6. Sivaraj A, Devi K, Vinothkumar P, Syed Zameer Ahmed K, Sathiyaraj K, David E, Senthilkumar B, " Lipid lowering effect of aqueous extract of flower of *Cassia auriculata*on streptozotocin (STZ) induced diabetic male *albino* rats", *Journal of Pharmacy Research*, 3(4): 683-686, (2010)
7. Nagappa AN, Thakurdesai PA,VenkatRao N, Jiwan Singh"Antidiabetic activity of *Terminaliacatappa*Linn fruits", *J. Ethnopharmacol*, (88): 45-50, (2003)
8. Pari, L. and Uma Maheswari, J. Antihyperglycaemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation in alloxan diabetic rats. *J.Ethnopharmacol*, 14: 136-138, (2000)
9. Syed Zameer Ahmed K, Sivaraj A, Vinothkumar P, Sundaresana S, Natarajan A, Devi K, Abdul Rahuman A, Senthilkumar B," Antihyperglycemic and antihyperlipidemic effect of ethyl acetate flower extract of *Cassia auriculata*on alloxan induced diabetes in male *albino* rats", *Journal of Pharmacy Research*, 3(6):1300-1303, (2010)

10. Muthulakshmi, A., Joshibhi Margret, R., Mohan, V.R. GC-MS analysis of bioactive components of *Feronia elephantum correa* (Rutaceae). *App.Pharmac. Sci*, 2: 69-74, (2012)
11. Kumar, B.D., Mitra, A., Manjunatha, M. In vitro and in vivo studies of antidiabetic Indian medicinal plants: a review. *J. Herbal Med Toxi*, 3: 9-14, (2009)
12. Rajkumar, L., Srinivasan, N., Balasubramanian, K. and Govindarajulu, P. Increased degradation of dermal collagen in diabetic rats. *Indian J. Exp. Bio*, 29: 1081-1083, (1991)
13. Item Justin Atangwhoa, Patrick Ekong Ebonga, Eyong Ubana Eyonga, Mohd Zaini Asmawib, Mariam Ahmadb. Synergistic antidiabetic activity of Vernonia amygdalina and Azadirachta indica: Biochemical effects and possible mechanism. *J. of Ethnopharmacology*, 141: 878– 887, (2012)
14. Puri, D., Prabhu, K.M. and Murthy, P.S. Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. *Indian J. Physiol. Pharmacol*, 46: 457–462, (2002)
15. Yadav, U.C.S., Moorthy, K. and Baquer, N.Z. Combined Treatment of Sodium orthovanadate and *Momordica charantia* fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. *Mol. Cell. Biochem*, 268: 111–120, (2005)
16. Yadav, U.C.S., Moorthy, K., and Baquer, N.Z. Effects of sodium orthovanadate and *Trigonella foenum graecum* seeds on hepatic and renal lipogenic enzymes and lipid profile during alloxan diabetes. *J. Biosci*, 29:81–91, (2004)