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Fabrication and Evaluation of Gastro-Retentive, Mucoadhesive Tablets from *Murraya Koenigii* Leaves

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ABSTRACT

Target drug delivery is beneficial for the delivery of pharmaceutical product to its appropriate site and with the resurgence in the use of herbal therapies as health care medication and this new field of drug delivery holds intensive research. The purpose of the current study was to design, gastroretentive mucoadhesive tablets using powdered leaves of *Murraya koenigii* and to optimize a product using natural gums and their combinations. The gastroretentive, boiadhesive drug delivery prolongs the residence time of the dosage form at the site of absorption and facilitates an intimate contact of the dosage form with the underline absorption surface and thus contribute to improve and/or better therapeutic performance of the drug and shows promising future in enhancing the bioavailability and specific needs by utilizing the physiochemical characters of both the dosage form and the mucosal lining Gastroretentive, mucoadhesive tablets using powdered leaves of *Murraya koenigii* were prepared using direct compression method and evaluated for parameters such as Weight variation, Hardness, Friability, Drug content, Swelling index, *In –vitro* drug release study, *In – vitro* and *In – vivo* mucoadhesive strength. Different types of natural gums such as Carbopol, Hydroxypropyl methylcellulose (HPMC) and a gas – generating agent (Sodium bicarbonate) were used. The investigation shows that the tablet composition and mechanical strength have the greatest influence on the floating properties and the drug release. With the incorporation of a gas – generating agent, along with the polymers, increased optimum floating (floating lag time to 30 minutes, and the duration of floating > 8 hours). The drug release was also increased and was sufficiently sustained (more than 8 hours) and non –Fickian transport of the drug was confirmed.

Keywords: Gastroretentive, mucoadhesive, *Murraya koenigii*, natural gums.

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INTRODUCTION

With the populace becoming more and more aware of the adverse effects of allopathic drugs, the ritual "pill for every ill" might be displaced by a demand that drugs be used only when essential. To overcome this, traditional medicines (TM) and the complementary and alternate medicines (CAM) have attracted more attention within the context of healthcare provision and health care. Thus in recent years, there has been resurgence in the use of herbal therapies and they are becoming increasingly popular in the general population. Millions of people today use herbal therapies along with prescription and nonprescription medicaments. Several factors which contribute to the increased use of herbal products are easy accessibility, perception of herbs as safe, alternate treatment, desire for self-medication and lesser cost. Today when we look around, the modern sedentary lifestyle with increasing stress, shifting food habits, we observe that there has been an increase in the number of diabetic patients and in fact due to this, India is known as the diabetic capital of the world. The World Health Organization (WHO) has also recommended the evaluation of plants effectiveness in conditions where we lack safe modern drugs.[1] With this there has been an increasing demand of research on antidiabetic natural products which produce minimal or no side effects[2] with a thought that a combination system of medication proves more beneficial than depending entirely on any one system. Diabetes Mellitus (DM) is one of the World's oldest diseases, and is mentioned in the Ayurvedic textbooks as "Madhumeha "meaning sweet urine. It is an endocrine disorder that is characterized by hyperglycemia[3] and altered metabolism of carbohydrates, lipids and proteins. It is caused by inherited and /or acquired deficiency in production of insulin by beta cells of pancreas by the ineffectiveness of the insulin produced, which leads to hyperglycemia and at a later stage lipid metabolism is also affected. The Non – insulin dependent diabetes mellitus (NDDM) is a multifactorial disease which is characterized by hyperglycemia and lipoprotein abnormalities.[4] These traits are hypothesized to damage cell membranes, which result in excess generation of reactive oxygen species. NDDM has also been associated with an increased risk for developing premature atherosclerosis due to an increase in triglycerides (TG) and low density lipoproteins (LDL) and increase in high protein lipoprotein (HDL)levels.[5] Presently ,two groups of oral hypoglycemic drugs , sulphonylurea and biguanides are used in the treatment of DM. They act by lowering blood glucose thereby delaying or preventing the onset of diabetic complications.[6] However their toxic side effects and sometimes diminution in response after prolonged use are problematic. A number of investigations of oral antihyperglycemic agents from plants in traditional medicines have been conducted and many

plants were found with good activity.[7, 8.] One such plant, *Murraya koenigii (Rutaceae)* has always been considered by the Indian traditional practitioners to have both preventive and curative activity against diabetes. Many workers had shown that the leaves of *M. koenigii* indeed have active antidiabetic properties in their animal models. Its antidiabetic properties are expressed at various levels. Bhat M et al[9] showed that it has significant alpha-amylase inhibitory property thus helping in preventing the sudden surge in glycaemia. Ponnusamy et al[10] found that their isopropanol extract had similar inhibitory effects on the enzyme. Khan et al[11] attributed their hypoglycaemic activity of *M. koenigii* to be due to increased glycogenesis and decrease glycogenolysis and gluconeogenesis in the liver of their rat models. This was evidenced by the increased activity of glycogen synthetase and decreased activity of glycogen phosphorylase and guconeogenic enzymes. Vinuthan[12] noticed that there was an increased in insulin activity in their alloxan-induced diabetic rats on the 43rd and 58th days of treatment with aqueous and methanol extract. They postulated that this effect could be due to either stimulation of insulin synthesis and/or secretion from the beta cells of pancreatic islets of Langerhans. Arulselvan[13, 14] concurred with this and went on to say that the antioxidant defence system was responsible for this effect by decreasing oxidative stress and pancreatic beta-cell damage. Yadav[15] found that curry leaves did not reduce blood glucose levels in normal rats as it does in mild to moderate diabetic rats. However, Kesari found this to be untrue in their rabbit model.[16] Another plus point for *M. koenigii* is its ability to control cholesterol levels as evidenced in studies done by Kesari,[8] Lawal[17] and Birari.[18.]Percent contributions of diabetically important elements from curry leaves were 1 – 2 % of daily dietary intake (DDI) but are likely to be in bioavailable form, thus making them effective for the treatment of diabetes. Rb and Cs are linearly correlated (r = 0.95) as their salts enhance the absorption of insulin in the lower respiratory tract by breakdown of glucose. Inorganic elements may remain complexed with organic ligands.[19] Although the leaves are used in curry, chutney and other condiment, many diabetic patients are not comfortable in the dietary consumption of the leaf. This problem can be solved by consideration of a suitable dosage form for administration of the plant leaves to enhance the combination drug treatment. The recent development in Novel Drug Delivery System (NDDS) aim to form a suitable dosage form for the administration and to target the drug delivery at its appropriate site. Bioadhesion is a topic of current interest in the design of drug delivery systems. One such approach is Gastro-retentive drug delivery system (GRDDS) that can be retained in the stomach for a prolonged period of time, thus ensuring its optimal bioavailability. It prolongs the residence time of the dosage form at the site of absorption and facilitates an intimate contact of the dosage form with the underline absorption surface and thus contributes to improved and/or better

therapeutic performance of the drug. The process wetting of bioadhesion involving a polymeric drug delivery platform is a complex one that includes adsorption and interpenetration of polymer chains amongst various other processes. Out of the different types of GRDDS available, an attempt has been made to prepare Bio / muco adhesive system which binds to the gastric epithelial cell surface or mucin, and extend the gastro – retention time (GRT) by increasing the intimacy and duration of contact between the dosage form and the biological membrane.

MATERIAL AND METHODS

Materials

The stems and leaves of *Murraya koenigii* were collected from mature trees in and around Rourkela, Odisha. Carbopol 934 P, HPMC K 100, sodium bicarbonate and PVP K – 30 were purchased from Cosmo Chem. Pune, India , magnesium stearate and talc were purchased from S.D.Fine Chemicals, Mumbai, India, and isopropyl alcohol and hydrochloric acid were purchased from Merck Limited, India.

Formulation of tablets

The compositions of the designed formulations of the powdered leaves of *Murraya koenigii* are listed in Table 1 .Tablets were formulated by wet granulation process. Powdered leaves, carbopol 934 P (gelling agent), HPMC K 100 C (hydrophilic polymer) and sodium bicarbonate (gas generating agent) were weighed accurately and triturated in the mortar. The mixture was then granulated by PVP – K 30 solution (5 % w/v in isopropyl alcohol). The wet mas was semi – dried at 40 ˚ C in hot air oven for 20 minutes. Magnesium stearate (1 %) and talc (1 %) were added as lubricants and the tablets were compressed on single punch CADMACH tablet machine.

Table 1: Formulation of tablets

Ingredients	Composition (mg /tablet)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
***M.koenigii* leaf powder**	300	300	300	300	300	300	300	300	300
Carbopol 934 P	200	100	300	200	200	200	200	200	200
HPMC K 100	100	100	100	80	120	100	100	100	100
Sodium bicarbonate	60	60	60	60	60	60	60	50	60
PVP K – 30	15	15	15	15	15	15	15	15	15
Magnesium stearate	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Talc	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5

Evaluation of tablets

The following evaluation tests for the prepared tablets were performed.

Tablet Hardness: Six tablets of each formulation were tested for hardness using Monsanto tablet Hardness tester.

Weight Variation: Twenty tablets were selected at random and the average weight was determined. The individual weight was compared with average weight.

$$\textbf{Weight variation} = \frac{M_{20} - M}{M} \times 100$$

Tablet thickness: Six tablets of each formulation was examined for their thickness and diameter using vernier calipers and the mean thickness and diameter was calculated.

Friability: Friability of tablets (n = 10) was determined by using a Roche Friabilator (Electrolab, EFL Friabilator), Mumbai, India. 10 tablets were rotated at 25 rpm for 4 minutes. The tablets were reweighed after removal of fines (using No. 65 sieve) and the percentage of weight loss was calculated by the following equation.

$$\% \text{ Friability} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Tensile strength: Tensile strength for crushing (T *) was calculated using the following

Equation $(T^*) = \frac{2F}{\pi d t}$

Where F = crushing strength

d = diameter of tablet

t = thickness of tablet

Crushing load was measured by Monsanto Hardness Tester.

Drug content

20 tablets of each formulation were weighed and powdered. A quantity equivalent to 0.1 gm of the leaf powder was weighed accurately and shaken with 70 ml of 0.1 N HCl for 15 minutes and diluted to 100 ml with 0.1 N HCl and then filtered. 10 ml of the filtrate was diluted to 100 ml with 0.1 N HCl. 10 ml of this solution was further diluted to 100 ml with 0.1 N HCl and the absorbance of the resulting solution was measured at 258 µm.

Water Uptake study[20]

The swelling of the polymers can be measured by their ability to absorb water and swell. The water uptake study of the tablet was done using USP dissolution apparatus II. The medium used was 900 ml of distilled water maintained at 37 ˚ C ± 0.5 throughout the study. After a selected time intervals, the tablet was withdrawn, blotted to remove excess water and weighed. Swelling characteristics of the tablets were expressed in terms of water uptake. (WU)

$$\text{WU \%} = \frac{(\text{weight of the swollen tablet} - \text{initial weight of the tablet})}{\text{Initial weight of the tablet}} \times 100$$

***In –vitro* drug releases studies**

Dissolution study of the tablet was done for 12 hours in 900 ml of Hcl buffer (pH 1.2) using USP dissolution I apparatus at 100 rpm , maintaining the temperature of the medium at 37 ˚ C ± 0.5 through out the study. Aliquot of dissolution medium was withdrawn at specific time interval, filtered and the absorbance measured at 258 µm.

Floating lag time and duration of floating

The buoyancy lag time and the duration of buoyancy were determined in the USP dissolution II apparatus in acidic environment (Hcl buffer pH 1.2).

In – vitro bioadhesion study [21]

In –vitro bioadhesion studies were done using rabbit gastric mucosa. The gastric mucosa was used immediately after dissection for this study. The detachment force i.e. the force required for separating the tablet from the gastric mucosa surface was fixed to the outer surface of the bottom of 100 ml beaker with cynoacrylate and then placed in 1000 ml beaker. The phosphate buffer pH 7.4 was added into the beaker up to the upper surface of the gastric mucosa such that the media remains just above the mucosa. The tablet was fixed to the modified stainless steel pan with cynoacrylate adhesive. A pre – load of 50 gm was placed on the pan for 5 minutes (preload time) to establish preload bonding between tablets and gastric mucosa. The preload weight and preload time were kept constant for all formulations. After completion of the preload time, preload was removed from the pan and water was then added into the beaker from a syringe at a constant rate. The addition of water was stopped when the tablet detached from the gastric mucosa. The weight of water required to detach the tablet from gastric mucosa was noted. The mass in grams required to detach the tablet from the mucosal surface gave the measure of bioadhesive strength. Force of adhesion was calculated from the following formulae –

$$\text{Force of adhesion (N)} = \text{Bioadhesive strength} \times \frac{9.81}{1000}$$

$$\text{Bond strength (N /m}^2\text{)} = \frac{\text{Force of adhesion}}{\text{Disk surface area.}}$$

The bioadhesion strength was measured only in formulation F 1 and F9.

RESULT AND DISCUSSION

The mechanical strength of all the tablets of all the batches was evaluated by the hardness, tensile strength and friability testing. (Table 2)

Table 2: Evaluation of prepared tablets

Formulation no/	F1	F2	F3	F4	F5	F6	F7	F8	F9

Parameters									
Hardness (kg / cm^2)	7.33 ± 0.29	6.67 ± 0.29	8.17 ± 0.58	8.00 ± 0.50	8.33 ± 0.29	8.80 ± 0.09	7.83 ± 0.76	7.00 ± 0.5	6.67 ± 0.29
Weight Variation	(-) 1.0512 to 1.055	(-) 1.495 to 1.62	(-) 2.246 to 1.945	(-) 1.884 to 2.251	(-) 2.626 to 2.358	(-) 1.65 to 1.834	(-) 1.755 to 1.558	(-) 2.115 to 2.225	(-) 1.753 to 1.499
Tablet Thickness (cm)	0.58 ± 0.03	0.53 ± 0.03	0.63 ± 0.03	0.48 ± 0.03	0.50 ± 0.05	0.47 ± 0.03	0.55 ± 0.05	0.48 ± 0.03	0.58 ± 0.05
Tablet Diameter(cm)	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14
Fraibility	0.2967 ± 0.03	0.2674 ± 0.04	0.31 ± 0.06	0.42 ± 0.04	0.35± 0.05	0.43 ± 0.05	0.29 ± 0.12	0.40 ± 0.05	0.40 ± 0.06
Tensile Strength (kg/cm^2)	7.062	7.031	7.245	9.312	9.308	10.469	7.954	8.148	7.453
Drug content (%)	99.23 ± 0.14	99.05 ± 0.03	99.35 ± 0.12	99.221 ± 0.18	99.50 ±0.21	99.11 ± 0.10	99.45 ± 0.27	99.28 ± 0.18	99.12 ± 0.10
Duration of floating(hours)	12	12	12	12	12	12	12	12	12
Water uptake study	274.49 ± 4.06	230.62 ± 4.31	357.57 ± 2.5	249.59 ± 4.04	301 ± 4.56	174.53 ± 4.02	178.68 ± 3.36	268.45 ± 2.17	271.43 ± 3.14
% drug released in 1 hr	26.209 ± 1.28	29.81 ± 1.46	23.12 ± 0.98	26.02 ± 1.28	27.33 ± 1.58	18.32 ± 1.36	26.20 ± 1.28	28.12 ±1.32	24.14 1.41±
% drug released in 12 hrs	94.80 ± 1015	97.20 ± 4.42	90.05 ± 1.22	94.80 ± 1.15	95.10 ± 1.10	84.76 ± 1.55	94.86 ± 1.15	96.2 ± 1.38	93.45 ± 1.10

The average hardness of all the batches were in the range of 6.67 ± 0.29 to 8.83 ±0.29, which is necessary in case of gastro retentive tablets to withdraw the peristalsis movement of the gastric mucosa.

The weight variation for all the batches was found to be within the acceptable range (less than 5 %).

Friability of all the batches was found to be less than 1%.

The tensile strength of all the batches was in the range of 7.031 – 10.496 kg /cm ^{2} revealing good mechanical strength of the tablets.

The drug content of all the batches was found to be greater than 99.5 and less than 101 %.

The swelling of the polymers used (carbopol 934 P, HPMC K 100 M and PVP K -30) was determined by the water uptake of the tablets. The complete swelling was achieved by the end of 8 hours. There was a significant increase in swelling of tablets ($P < 0.005$) with increase in carbopol

934 P concentrations (F1 –F3). Similarly increase in concentrations of HPMC K 100 M also showed increase in swelling but not to that extent of carbopol 934 P. The reason could be comparatively lower concentration of HPMC K 100 M used for formulation development as compared to carbopol 934 P. The effect of swelling agent, PVP K – 30 was found to be significant ($P < 0.005$). No significant difference was observed on the swelling property by varying the concentration of sodium bicarbonate. The swelling polymers carbopol 934 P, HPMC K 100 M contribute to the swelling properties apart from the release retarding rate. Diffusion of the drug significantly depends on the water content of the tablet. This may be because of the motility of the polymer chains which strongly depends on the water content of the system. At high water content, in the polymer chains, relaxation takes place with expansion in volume of the system. This helps in the higher penetration of the gastric fluids into the tablet, leading to carbon dioxide gas generation and thus reducing the floating lag time. It also helps in increasing the tablet dimensions leading to increase in diffusion pathway to achieve complete release in 12 hours. (Figure 1, Table 3). Apart from the floating property of the tablet bioadhesive tendency could be an important property for gastro - retentive drug delivery. Both carbopol 934 P and HPMC K 100 M have potential bioadhesive property. The bioadhesive property was found to be significant ($P < 0.005$) increase when used in equal proportion combination (F 1) as compared to when used alone. The bioadhesive property of the polymers may be due to its physical or hydrogen bonding with the mucus components. This helps the tablets to stay in the upper part of the G.I.T. and enhance the gastro – retention along with the floating and swelling property (F 1). (Table 4)

Table 3 Cumulative % drug release with time

Time interval (hrs)	Cumulative % drug release	
	F 1	**F 9**
1	26.20 ± 1.28	24.14 ± 1.41
2	37.52 ± 1.45	35.15 ± 1.41
3	47.92 ± 1.36	44.53 ±. 1.33
4	57.42 ± 1.07	54.92 ± 1.35
6	70.48 ± 1.64	68.18 ± 1.65
8	82.48 ± 1.20	79.02 ± 1.42
12	94.80 ± 1.15	93.45 ± 1.10

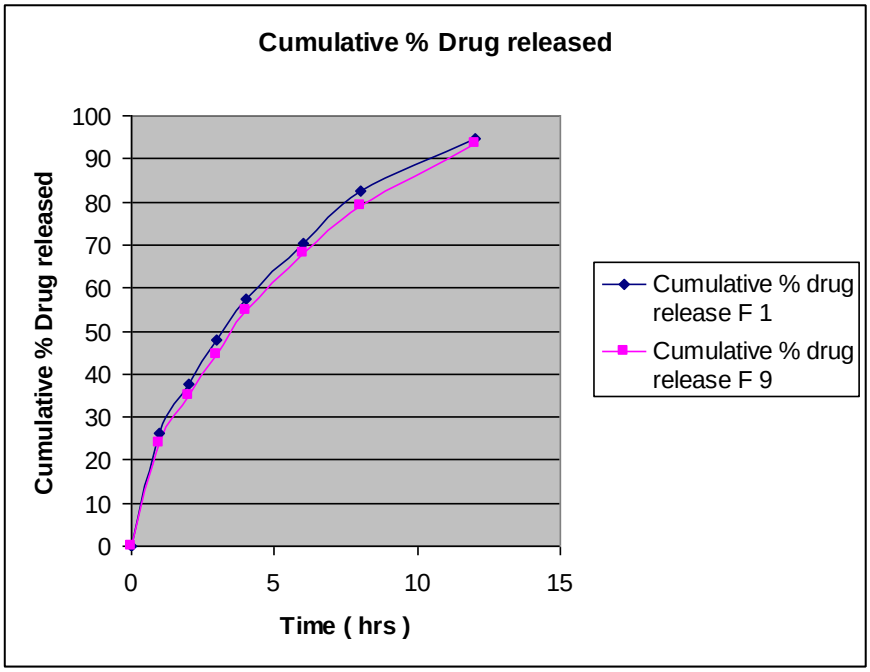

Figure 1 Cumulative % drug released

Table 4: Bioadhesive strength of selected formulations F1 and F9

Parameters	F 1	F 9
Bioadhesive strength (g)	13.8 ± 0.35	12.53 ± 0.61
Force of adhesion (N)	0.1354	0.1229
Bond strength (N /m^2)	331.75	301.22

CONCLUSION

Murraya koenigii leaves have good anti – diabetic reduction of blood sugar properties and if consumed daily shows significant results. Keeping in view that many patients are unable to consume the leaves due to its aroma, the present work to design a bioadhesive, gastro-retentive drug delivery system, with good floating and swelling properties and gastro –retention upto the desired period of time was undertaken. On the basis of *in – vitro* drug release, bioadhesion bond strength and floating lag time, formulations F1 and F9 were selected as the optimized formulations showing promising results.(Table 5). From the above results, the formulation F1 was concluded to be the final optimized formulation as it showed higher bioadhesive bond strength. Dissolution profile of both the batches were compared with one way ANOVA and showed statistically non – significant difference. Although the onset of floating was earlier in formulation F9 when compared with F 1 but the duration of floating was found to be same for both the formulations i.e. 12 hours. Higher bioadhesive bond strength would ensure superior gastro –retention of F 1 than F9.(Table 5). Hence it can be concluded that carbopol 934 P and HPMC K 100 M increases the dimensional

stability of the formulation which is necessary in case of sustained release formulation and a combination of carbopol 934 P, HPMC K 100 M and PVP K -30 can be a promising polymer for gastro –retentive drug delivery system. Sodium bicarbonate acts as a gas generating agent which imparts buoyancy in GRDDS. The optimized formulation followed Higuchi kinetics while the drug release mechanism was found to be anomalous type, controlled by diffusion through the swollen matrix. Swelling studies indicated significant water uptake which contributed in drug release. The polymer combination of carbopol 934 P and HPMC K 100 M also showed significant bioadhesion. Thus combining these approaches of gastro –retention together, the *in – vivo* gastro –retention could be predicted more reliably.

Table 5: Comparative values of different parameters for F 1 and F 9

Parameters	**F 1**	**F 9**
Hardness (kg)	7.33±0.29	6.67±0.29
Weight	Acceptable	Acceptable
Tablet thickness (cm)	0.58±0.03	0.50±0.5
Friability	0.2967±0.03	0.40±0.06
Tensile strength (kg /m^2)	7.06	7.453
Drug content (5)	99.23 ±0.14	99.12±0.1
Floating lag time (min.)	21.33±3.15	15.15± 3.0
Duration of floating (hrs)	12	12
% Swelling	274.49±4.06	271.43±3.14
% drug release(in 1 hr)	26.20±1.28	24.14±1.41
Cumulative % drug release (in 12 hrs)	94.80 ±1.15	93.45±1.10
R^2 forHiguchi equation	0.9928	0.9869
‘n’ value for Korsmeyer – peppas equation	0.5336	0.5476
Bioadhesive bond strength(N/m^2)	331.95	301.22

Future Scope

Based on these promising *in –vitro* results, *in – vivo* studies in animal models and healthy volunteers may be carried out for the determination of various pharmacokinetic parameters to make the gastro –retentive drug delivery system a promising drug delivery system for herbal products.

REFERENCES

1. Day C Traditional plant treatments for diabetic mellitus: Pharmaceutical foods, Br J Nutr 1998; 80: 5 - 6.
2. Rao BK, Kesavulu MM, Giri RA Antidiabetic and hypolipidemic effects of *Momondica cymbalaua* roots, fruit powder in alloxan induced diabetic rats. J Ethanopharmacol, 1999; 67: 103 – 9.

3. Chandra A, Singh RK, Tewari L. Antioxidative potential of herbal hypoglycemic agents in diabetes-an overview. SFRR-Indian Bulletin 2004; 3: 24–26.
4. Scoppria A, Monteechi FR, Mezinger G, Lala A. Urinary mevalonate excretion rate in type 2 diabetes: role of metabolic control. Alherosclersis. 2001; 156: 357 – 36
5. Belteridge DJ. Diabetic dyslipidemia. AmJ Med 1994; 96:525 -31.
6. Stephen N, Davis and Daryl K, Granner, Insulin, Oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In Goodman and Gilmans's The Pharmacological Basis of therapeutics 10th Ed. Hardman, J.G., Limbird, L.L. and Gilman, A.G.(eds),maGraw – Hill, Medical publishing division, New Delhi, 2001; 1701 -1709.
7. Marles RT, Franswottn NR Antidiabetic plants and their active constituents. Phytomedicine, 1995; 2: 137 - 89.
8. Kesari AN, Kesari S, Singh SK, Gupta RK, Watal G. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. J Ethnopharmacol. 2007; 112(2) : 305-11.
9. Bhat M , Zinjarde SS. , Bhargava S, Y. Kumar AR ,Joshi B N. Antidiabetic Indian Plants: A Good Source of Potent Amylase Inhibitors, Evidence-Based Complementary and Alternative Medicine 2011,1- 6.
10. Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Ravi Kumar A. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evid Based Complement Alternat Med. 2011; 2011. pii: 515647. Epub 2010 Sep 23.
11. Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard): mechanism of action. Indian J Biochem Biophys. 1995; 32(2):106-8.
12. Vinuthan MK, Girish Kumar V, Ravindra JP, Jayaprakash, Narayana K. Effect of extracts of *Murraya koenigii* leaves on the levels of blood glucose and plasma insulin in alloxan-induced diabetic rats. Indian J Physiol Pharmacol. 2004; 48(3):348-52.
13. Arulselvan P, Senthilkumar GP, Sathish Kumar D, Subramanian S. Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats. Pharmazie.2006; 61(10):874-7.
14. Arulselvan P, Subramanian SP. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats. Chem Biol Interact. 2007 ;165(2):155-64.

15. Yadav S, Vats V, Dhunnoo Y, Grover JK. Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. J Ethnopharmacol. 2002; 82(2-3):111-6.
16. Kesari AN, Gupta RK, Watal G. Hypoglycemic effects of *Murraya koenigii* on normal and alloxan-diabetic rabbits. J Ethnopharmacol. 2005; 97(2): 247-51.
17. Lawal HA, Atiku MK, Khelpai DG, Wannang NN. Hypoglycaemic and hypolipidaemic effect of aqueous leaf extract of *Murraya koenigii* in normal and alloxan-diabetic rats. Niger J Physiol Sci. 2008; 23(1-2): 37-40.
18. Birari R, Javia V, Bhutani KK. Antiobesity and lipid lowering effects of *Murraya koenigii* (L.) Spreng leaves extracts and mahanimbine on high fat diet induced obese rats. Fitoterapia. 2010 ; 81(8): 1129-33.
19. Choudhury RP, Garg AN Varition in essential, trace and toxic elemential contents in *Murraya koenigii* – Aspice and medicinal herb from different Indian states.Food Chemistry 2007; 104(4): 1454 – 1463.
20. Patil CM, Jain P, Chaudhari S, Shear R, Vavia PR Int j pharm 2006; 304: 86 -92.
21. Desai KGH, Kumar TMP AAPS PharmaSci Tech 2004; 5(3) Article 35 (http // www. Aaps pharmascitech.org).