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Original Article

# An Alternative Excipient from Vegetable Source for Oral Drug Dosage Forms to Regulate Drug Delivery

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## ABSTRACT

**Introduction:** Tablet is the preferred drug dosage form. Cellulose fiber (Micro Crystalline Cellulose) is the major excipient used. For chronic diseases, frequent dosage is required resulting in cumulative excipient load and tachyphylaxis due to drug. We intent to invent excipients from vegetable sources to mitigate above challenges and reduce dosage as chronic sufferers (Diabetes mellitus) requires lifelong treatment. **Aims and Objectives:** To develop an excipient from *Brassica oleracea* and *Vigna radiata* sprouting which can regulate drug delivery, avoid excipient led toxicity and tachyphylaxis. **Materials and Methods:** Alkali and acid based depolymerization method was followed and cellulose fibers were studied for safety, physical, chemical and molecular parameters. With the developed cellulose fibers two drugs were formulated using Active Pharmaceutical Ingredient- Metformin and Miglitol. Efficacy was assayed using cell lines-Neuroblastoma, Kidney HK-2, L6 Myoblasts, 3T3-L1 Preadipocytes, INS-1 and HEPG 2 hepatocytes and compared with conventional micro crystalline cellulose- based formulations. **Results:** Characterization of developed excipients was done in comparison with conventional cellulose, invented excipients had long fibers, higher bulk density, flow, even particle size compared to conventional cellulose. Dissolution and disintegration of Metformin from *Brassica oleracea* fiber was slow and Miglitol showed quick release from *Vigna radiata* compared to conventional excipient. Therapeutic effect of both drugs from developed excipients was higher compared to conventional excipient-based drug by cell culture assay. Both developed excipients did not show mutagenic effect. **Conclusion:** *Brassica oleracea* fiber slows release of metformin; may reduce drug dosage, tachyphylaxis, release of Miglitol from *Vigna radiata* was rapid, may compliment metformin therapy.

**Keywords:** Herbal excipients, Cellulose fibers, Microcrystalline cellulose, Tachyphylaxis, Vegetable source excipient, *Brassica oleracea*, *Vigna radiata*, Metformin, Miglitol.

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## INTRODUCTION

Among various dosage forms of drugs in the allopathic system of medicine, tablet dosage form is the most preferred form of drug because of portability comfort and high degree of compliance scope by the patient.[1] Chronic diseases like psoriasis, diabetes mellitus, arthritis etc., requires lifelong medication with high frequency of drug intervention. Such kind of medication often would result in drug resistance by the system called as tachyphylaxis.[2] Besides the above, the excipient(s) of the tablet due to frequent drug usage also would produce their share of side-effects at cumulative level.[3] If time-release of the drug is achieved, the enhancement of the therapeutic value as well as dosage reduction, both can be achieved.[4] Thereby the problem of tachyphylaxis and excipient lead toxicity can be avoided.

Metformin is the most sought-after prescription drug for treating type 2 diabetes mellitus. Metformin exerts an array of pharmacological effects resulting in improved sensitivity of cells towards insulin. The frequency and high dosage of metformin is required when the blood glucose level is too high which often results in various metformin related complications, especially Vitamin $B_{12}$ depletion.[5] Sustained release metformin of 850 mg is already available which helps to reduce the daily dosage of 1000 mg, wherever such high dosage is required. If metformin is engineered to release slow from the base (excipient) in a programmed/ sustained manner, the efficacy potentiation can be easily achieved along with dosage reduction. But as on date, a backbone excipient with 'extra' nutritional value that can replace the conventional microcrystalline cellulose (MCC) is far from reach.

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Other pharmaceutical agents used for the treatment of type II DM are combination of Miglitol and Metformin which essentially inhibit the enzyme that is responsible for the conversion of carbohydrate to glucose (α- glucosidase). With reference to the release per se of the above two drugs, we presume slow release of metformin and rapid release of miglitol may offer better therapeutic benefit to the patient.

In the present study, we have explored the scope of developing an excipient, alternative to MCC from *Brassica oleracea* (Bracoli) and *Vigna radiata* (moongdal) sprouts. We chose the above source due to rich digestible fiber contents presence, nutritional value and known safety. We have adopted the conventional acid and alkali based depolymerization method, followed by purification with $H_2O_2$ for obtaining cellulose fibers from the above plants.

Using the cellulose fiber thus obtained, both Metformin and Miglitol were formulated and tested by cell culture method using Neuroblastoma, Kidney HK-2, L6 Myoblasts, 3T3-L1 Preadipocytes, INS-1 and HepG2 hepatocytes. The formulation of the above drugs of the same dosage was also made with MCC and compared the activity. Sustained release metformin solid dosage form of the same strength was used for comparison.

The physio-chemical characteristics of the depolymerized cellulose fibre obtained from *Brassica oleracea* and *Vigna radiata* sprouts were studied in comparison with MCC using standard method.[6-10] Details of the findings are presented in the article.

## MATERIALS AND METHODS

### Preparation of depolymerized cellulose fiber from *Brassica oleracea* (Broccoli)

Fresh, healthy *Brassica oleracea* (Broccoli) was obtained and then was washed thrice, cut into small pieces to remove the stem portions and then used. Then the plant material was then treated with 50-70% NaOH at 70°C for 2 hr and then the fiber was cleaned thoroughly using water. The dried fiber was then bleached with 40% $H_2O_2$ three times and then washed with water. The thus obtained fiber was neutralized with 10N HCl and washed with water to remove the residual acid, air dried and stored in airtight container until use.

### Preparation of depolymerized cellulose fiber from *Vigna radiata* (Mung bean) sprout

The *Vigna radiata* (Mung bean) were soaked overnight in distilled water to imbibe sufficient water and then were allowed to sprout in a cloth bag stored inside an aerated, temperature and humidity adjusted, dark chamber. Adequate care was taken to moisten the seeds regularly to facilitate sprouting. On day 7, the sprouts were separated from cotyledon shell and subjected depolymerization process as described above.

### Test sample preparation for cell culture assay

We followed the method described by Aruna *et al*., 2022 for the present study.[11] In brief, Metformin 500 mg sustained release tablet (uncoated) available in the market was used as reference test sample for the present study. 500 mg metformin active pure compound was formulated as tablet using partially depolymerized cellulose fiber of *Brassica oleracea* and *Vigna radiata* sprouts separately without any other additional excipient.

Similarly, 500 metformin was also formulated with MCC without the addition of any other excipient. The tablet formulations of Metformin 500 mg were dipped into 50 mL of Phosphate buffer at different time intervals such as 0 hr, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 7 hr and 9 hr. After which, the mixture was centrifuged and the 10µL of the sample from each set was used for cell culture assay where the objective was to study the effective release time versus efficacy. For cytotoxic study, 10, 50 and 100 µg/mL of the test sample was used.

Similarly, the release of metformin from different formulations was measured using a spectrophotometer at 532 nm and concentration was plotted against standard curve of metformin (pure compound) in phosphate buffer.

### Cell culture assay

Minimal essential medium (MEM) was used for the study. Supplements such as 1mM non- essential amino acids, 0.5 mM L-glutamine, 0.1 mM sodium pyruvate, FBS 10% were used. For the present study SK N SH human neuroblastoma cells were used. The above cells in the said medium was incubated in 5% $CO_2$ chamber at 37°C to allow the cells to reach 80% confluence.

### Cytotoxicity assay

MTT assay was performed to study the cytotoxic effect of vegetable based cellulose fiber.[12] In brief, the cultured SK N SH cells ($0.2X10^6$ cells per well) were seeded into 96-wellplate containing in 200 mL of medium supplemented with 10% FBS and then exposed to varying concentrations of the test samples ranging from 10 to 100 µg/mL. After 24 hr of treatment, MTT reagent (20µL) was added and incubated for further 4 hr period. The formazan thus formed was dissolved in 200 µL of 0.1 N acidic isopropyl alcohol and read at 570nm.

### Neuroprotection assay

The method described by Nistico *et al.*, 2008 was followed in the present study; where the neural cell - SK N SH was subjected to stress.[13] For this purpose 1.0 mM of $H_2O_2$ was used. One set of cells were treated with the test samples at concentration ranging from 10 to 100 µL/mL and then incubated with MTT for 3hr in a humidified $CO_2$ incubator with 5% $CO_2$. The test samples pre-treated cells after 24hr, were treated with 1.0 mM $H_2O_2$ to induce cell death, cell incubated for 24 hr. After incubation,

MTT assay was performed and results of test samples treated and untreated were compared.

## Study on Kidney HK-2 cells

The present study was performed in line with the earlier work of Ryan *et al.*, 1994.[14] In brief, Keratinocyte SFM medium with 5% FBS along with rhEGF 0.005g/mL, bovine pituitary extract 0.05 mg/mL. the above setup was incubated in 5% $CO_2$ chamber at 37°C. Tryphan blue dye exclusion assay was performed over $2X10^4$ cells. The cells were treated with the test samples as described above. MTT assay was performed to understand the cytotoxic effect of the test samples.

## Triglyceride (TG) measurement in HepG2 cells

Triglyceride measurement in HepG2 cells was performed by adopting the method of Xiaopeng Zhu *et al.*, 2018.[15] One group of HepG2 cells were treated with 30 mM of glucose and 100 nM of Insulin. To the other set of cells, in addition to the above treatment, 10 µL of test samples was also added.

TG was evaluated by digesting the PBS washed cells in trypsin (300 µL) for 2 min and then 700 µL of fresh medium was added to stop further digestion. From the total of 1000µL sample, 100 µL was used for protein quantification and rest of the 900 µL was used for TG measurement. The portion meant for TG measurement was centrifuged at 800 rpm for 3 min, then 1 mL of chloroform/methanol (2:1 v/v) was added and vortexed for 2 hr. Then 500 µL of 0.1 M Nacl was added, centrifuged at 3700 rpm and then the lower layer was transformed, dried, treated with 40 µL of 1% Triton X, and TG was measured using the TG reagent kit.

## Assay of Glucose utilization in HepG2 cells

We have adopted the method of Kerimi *et al.*, 2015 for the study.[16] In brief, HepG2 cells were dislodged from tissue culture flask using 0.25% trypsin phosphate buffered saline, the cell density of 6000 cells per well was loaded in 96 well culture plate and incubated as described elsewhere. The media was changed on day 3, then the test material was added. After 2 days the medium was removed and replaced with 25 µL incubation buffer which is RPMI with PBS 0.1% BSA and 8 mM glucose. The setup was again incubated for 3 hr under the same condition followed for the cell culture.

1,1-Dimethylbiguanide hydrochloride (0.1 gm/mL) was used as positive control and without the same used as negative control. Subsequently, 10 µL of medium was transferred and incubated with 200 µL of glucose oxidase reagent to measure the concentration of glucose in the medium. After 15 min, absorbance value was taken at 492 nm using micro plate reader, the glucose utilized by the cells was calculated.

## Glucose utilization by L6 Myoblasts

The method of Yap *et al.*, 2007 was followed for the present study.[17] In brief, L6 Myoblasts 3000 cells/well were plated into 96 well culture plate with DMEM containing 2% FBS and incubated for 5 days. 48 hr prior to the incubation period, the medium was replaced and insulin 4g/mL instead of test sample in one row, and along with test sample was added wherein he incubation buffer was RPMI with PBS, 0.1% BSA and 8 mM glucose. Incubation of the same for 3 hr at the said condition was followed, after which 5 µL of incubation buffer was loaded into 96 well plate and then reacted with 200 µL of glucose oxidase reagent. Absorbance after 15 min incubation was done at 520 nm using micro titre plate reader. The glucose utilized by the control cells, test drug treated cells and insulin treated cells were calculated.

## Lipid Accumulation in 3T3-L1 Preadipocytes

The methodology of Park and Sung, 2015 was followed in the present study.[18] 3T3-L1 Preadipocytes cells at the density of 6000 cells /well was plated into 48 well culture plate. After 2 days the cells were treated by the test samples and Rosiglitazone 0.4 gm/mL, used as positive control. Then the cells were grown in DMEM with 10% FBS for 10 days with media replacement followed at every 2-3 days. After 10 days the cells were washed with PBS and allowed to fix at room temperature with 500 µL per well 10% formaldehyde in PBS. The fixative solution was removed and cells were stained with 200 µL pre-warmed oil red solution and incubated for 15 min. After which excess dye was washed and dried at 37°C. Then the dye was extracted in iso-proponal and then 200 µL of the solution was read at 520 nm using micro titre plate reader.

## Glucose metabolism by INS-1 cells

This test was performed to determine insulin secreting ability of INS-1 cells which was done using standard procedure.[19] RPMI medium with 5% FCS was used. 8000 cells per well was seeded and MTT assay was performed. The INS-1 cells were cultured in RPMI containing 5% FCS. The cells were seeded into 96-well plates at a density of 8000 cells per well, with a volume of 100 µL. The cells were left to attach overnight and treated with the test drugs prepared as described above or PBS (which serve as a control) in the presence or absence of glucose (20 mM). After 48 hr of incubation at 37°C, medium was removed from the cells and 100 µL of DMEM medium containing 10% FCS and 0.5 mg/mL M was added and incubated for additional 30 min at 37°C. The medium was later aspirated and MTT crystal (purple formazan) was dissolved in DMSO (200 µL/well). The absorbance was read at 540 nm using a microplate reader.

## Alpha glucosidase assay

According to the method described in the work of Amruthavalli *et al.*, 2019 was followed in the present study.[20] In a 96- well plate reader, a reaction mixture containing 50 µL of phosphate buffer

(50 mM; pH 6.8), 10 µL of alpha-glucosidase (1 IU/mL) and 20µL of test sample (miglitol) was incubated for 5 min at 37°C, and then 20 µL of 1 mM PNPG was added to the mixture as a substrate. After further incubation at 37°C for 30 min, the reaction was stopped by adding 50 µL of sodium carbonate (0.1 M). The enzyme, inhibitor and substrate solutions were made using the same buffer. Voglibose was used as a positive control and distilled water as negative control. The yellow colour produced (due to pnitrophenol formation) was quantitated by colorimetric analysis and reading the absorbance at 405 nm.

The % inhibition calculated using the formula: % inhibition = {Absorbance (control) –Absorbance (sample)}/ Absorbance (control) $IC_{50}$ value is defined as the concentration of extract inhibiting 50% of alpha-glucosidase activity under the stated assay conditions.

## RESULTS

### Yield

Two hundred gram of broccoli of two different batches were taken for partial depolymerization. After reducing the moisture level to 5% by drying, we got the yield of 120/200 and 141/200 g respectively for two batches. After subjecting partial depolymerisation process on the above fiber, the final yield obtained was 102/120 and 121/141 gm with the percentage final yield of 85 and 86 respectively for the two batches Table 1.

Two hundred gram of Mung bean sprouting of two different batches were taken for partial depolymerization. After drying to attain 5% moisture level, we got the yield of 76/200 and 81/200 gm respectively for the two batches. After subjecting the above fiber for partial depolymerisation process, the final yield obtained was 65/76 and 67/81 gm with the percentage final yield of 85 and 83 respectively for the two batches Table 2.

The broccoli fiber after first cycle of treatment for de-polymerization appeared deep yellow in colour and on third cycle of treatment appeared near white with increased fineness, high flow property and powdery-ness. In the case of Mung bean sprouting also, three cycles of treatment for partial de-polymerization has distinctly reduced the colour from deep yellow to near white with significant improvement in flow property, fineness and powdery-ness Table 3.

### Powder microscopy

#### *Microscopic appearance of partially depolymerized cellulose fiber -Figure 1, 2 –Vigna radiata (Mung bean) Sprouting*

Varying degrees of length, breadth and orientation of the fibres of Mung bean sprouting was observed after third cycle of treatment as in Figure 1 and 2.

### Microscopic appearance of partially polymerized cellulose fiber -Figure 3, 4 –*Brassica oleracea* (Broccoli)

Three cycles of treatment of Braccoli fibres for partial depolymerization, we obtained flat, globoid, irregularly shaped, deep with hyaline receptacles and occasionally elongated fibres as in Figure 3 and 4. Highly clustered, clumsy, irregularly shaped fiber with enlarged, flat deeply coloured fibres were observed when MCC was examined under a microscope as in Figure 5 and 6.

### Microscopic characterization of depolymerized cellulose fiber–Micro crystalline cellulose (MCC)

#### *FTIR spectra*

The FTRI spectra of the partially de-polymerized Braccoli fibres showed a distinct pattern with a few comparable peaks with that of MCC between 1000 to 3500 wave number as shown in Figure 7. The FTIR spectra of Mung bean sprouting showed highly comparable pattern with that of MCC where MCC showed a strong band at 3390 $cm^{-1}$ and a band at 1636 $cm^{-1}$ corresponding to the stretching and bending modes of the surface hydroxyls of MCC. The peak at 2905 $cm^{-1}$ belongs to the asymmetrically stretching vibration of C-H in a paranoid ring, and broad absorption peak 1059 $cm^{-1}$ is attributed to the C-O of cellulose, mung bean showed similar FTIR patter of MCC as in Figure 8. The FTIR pattern of MCC showed a strong band at 3390 $cm^{-1}$ and a band at 1636 $cm^{-1}$ corresponding to the stretching and bending modes of the surface hydroxyls of MCC. The peak at 2905 $cm^{-1}$ belongs to the asymmetrically stretching vibration of C-H in a paranoid ring, and broad absorption peak 1059 $cm^{-1}$ is attributed to the C-O of cellulose as in Figure 9.

### Ash content analysis

The ash content analysis has revealed insignificant presence of ash content in the partially depolymerized fibres of both Broccoli and Mung bean sprouting when compared to the significantly high level of ash in MCC, Table 4.

### Particle density test

The bulk density and tapped density of the partially depolymerized fibres of Broccoli and Mung bean sprouting were relatively higher than that of MCC and so was the Hausner's ratio of the above in comparison with MCC, Table 5.

### Angle of repose

The evaluation of angle of repose study of the partially depolymerized fibres of Broccoli and Mung bean sprouting were highly comparable with the angle of repose of MCC meeting similar flow property, Table 6.

**Table 1:** Yield of *Brassica oleracea* (Broccoli).

| Batch details | Initial weight of wet *Brassica oleracea* (Broccoli) (g) | After air drying (moisture level 5%) | After partial de-polymerization gm. and (%) |
|---|---|---|---|
| 1 | 200 | 120 | 102 (85) |
| 2 | 200 | 141 | 121 (86) |

**Table 2:** Yield of *Vigna radiata* (Mung bean).

| Batch details | Initial weight of wet *Vigna radiata* (Mung bean) (g) | After shred drying (moisture level 5%) | After partial de-polymerization gm. and (%) |
|---|---|---|---|
| 1 | 200 | 76 | 65 (85.5) |
| 2 | 200 | 81 | 67 (82.71) |

**Table 3:** Physical characteristics of partially de-polymerization.

| Herb | Parameters | First cycle | Second cycle | Third cycle |
|---|---|---|---|---|
| *Brassica oleracea* (Broccoli) | Colour | Deep yellow | Pale yellow | Near white |
| | Fineness | Corse | Corse | Very fine |
| | Powderyness | Fibrous | Less fibrous | Powdery |
| | Flow property | Poor | Poor | High |
| *Vigna radiata* (Mung bean) Sprouting | Colour | Pale yellow | Pale yellow | Near white |
| | Fineness | Corse | Corse | Very fine |
| | Powdery ness | Fibrous | Less fibrous | Powdery |
| | Flow property | Poor | Poor | High |

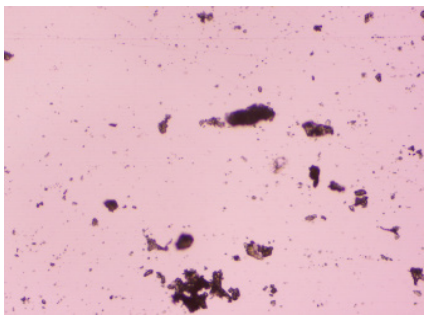

**Figure 1:** *Vigna radiata* fibers in 4X.

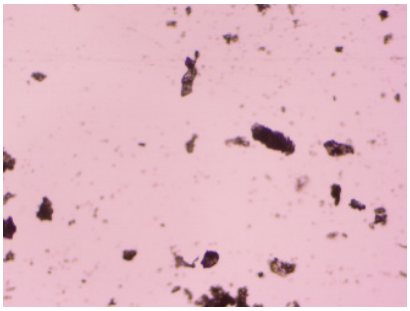

**Figure 2:** *Vigna radiata* fibers in 10X.

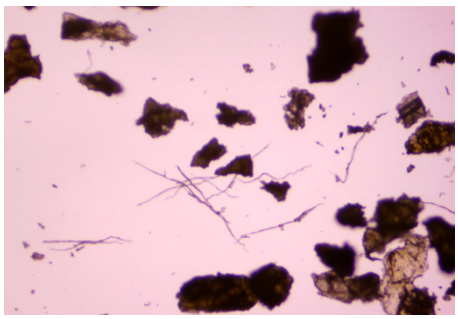

**Figure 3:** *Brassica oleracea* fibers in 4X.

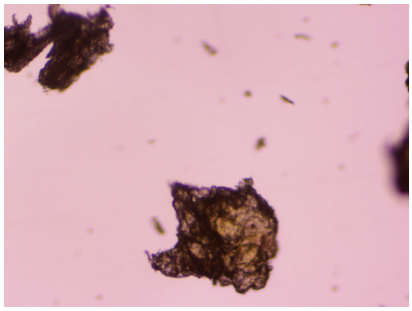

**Figure 4:** *Brassica oleracea* fibers in 10X.

## Formulation development

Five tablet formulations were made with the active pharmaceutical drugs such as metformin and Miglitol. Metformin 500 mg tablet was formulated separately with 50 mg each of MCC, the partially depolymerized Broccoli fibres and partially depolymerized Mung bean sprouting. Similarly, 20 mg of Miglitol was made into tablet using 50mg of MCC, partially depolymerized fibres of Broccoli and Mung bean sprouting, Table 7.

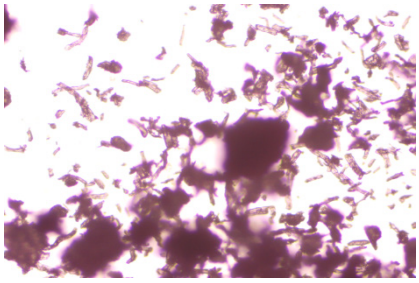

**Figure 5:** MCC in 4 x.

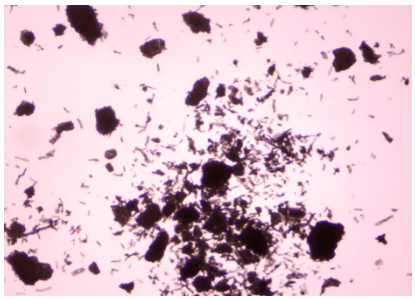

**Figure 6:** MCC in 10X.

## Evaluation of tablet formulations

The tablet formulations of 500 mg Metformin and 20 mg Miglitol using 50 mg each of partially depolymerized fibres of Broccoli and Mung bean sprouting showed highly comparable physical characteristics with reference to surface finish of the tablet, hardness, friability and disintegration time with that of the tablet formulated with MCC, Table 8.

## Cell line studies

None of the four formulations of Metformin exhibited strong cytotoxic effect on SKNSH human neuroblastoma cells from the concentration ranging from 10 to 100 µL. However, the Metformin sustained release and Metformin formulated in MCC showed a slight increase in cytotoxic effect on SKNSH human neuroblastoma cells vis-à-vis concentration when compared to the formulation of Metformin in partially depolymerized fibres of Braccoli and Mung dal sprouting suggesting faster release of drug from MCC and sustained release formulation Table 9.

Study on Neuroprotection effect of the four formulations of Metformin after $H_2O_2$ induction, the cells showed that Metformin formulated in depolymerized fibres of Broccoli offered significant protection (63%) at 100 µL whereas all the other three formulations did not offer such protection vis-à-vis concentration. When compared to control, all the four formulations of Metformin offered good degree of Neuroprotection, Table 10.

The cytotoxic study on kidney cells HK2 of the four different formulations of Metformin showed that the formulation in partially depolymerized fibres of Broccoli from 10 to 100 µL did not cause any cytotoxic effect whereas other three formulations showed a small linearity in cytotoxic effect, however the effect was not significant, Table 11.

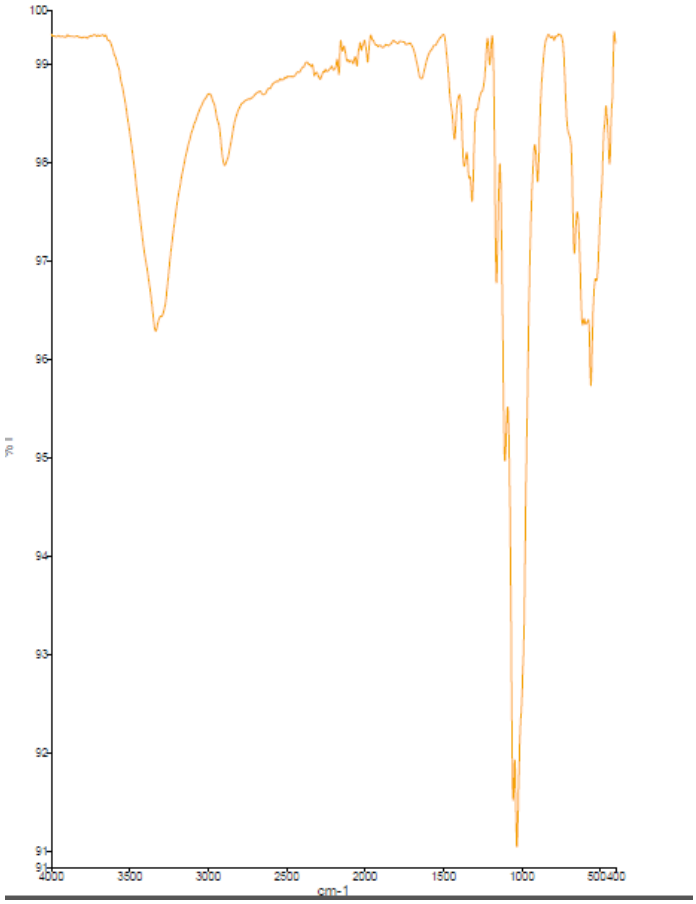


**Figure 7:** FTIR spectra of *B. oleracea* fibers.

Triglyceride accumulation study showed that the cells treated with high glucose and high insulin showed TG accumulation of 62 µg. The Metformin formulated in partially depolymerized Braccoli fiber did not induce TG accumulation vis-à-vis the release time of 9 hr. Whereas the other two formulations of Metformin although showed a slight increase in TG accumulation in HepG2 cells vis-à-vis time of drug release, however the TG accumulation was not significant, Table 12.

Glucose utilization by HepG2 cells treated with the four different formulations of Metformin showed that the drug formulated in partially depolymerized Braccoli fiber augured greater glucose utilization by cells vis-à-vis release time suggesting sustained higher release of drug over time may be responsible for the above effect when compared to other three formulations. Appreciable linearity between level of glucose utilization and time of drug release from all the formulations was also observed, Table 13.

The glucose utilization by L6 Myoblast cells treated with the four formulations of Metformin showed that Metformin formulated with partially depolymerized fiber of Braccoli significantly accelerated glucose utilization by the cells when compared to other three formulations of Metformin. However, other three formulations also showed positive effect on the cells with reference to glucose utilization. Release time vis-a-vis activity linearity was also observed in the case of Metformin formulated with partially

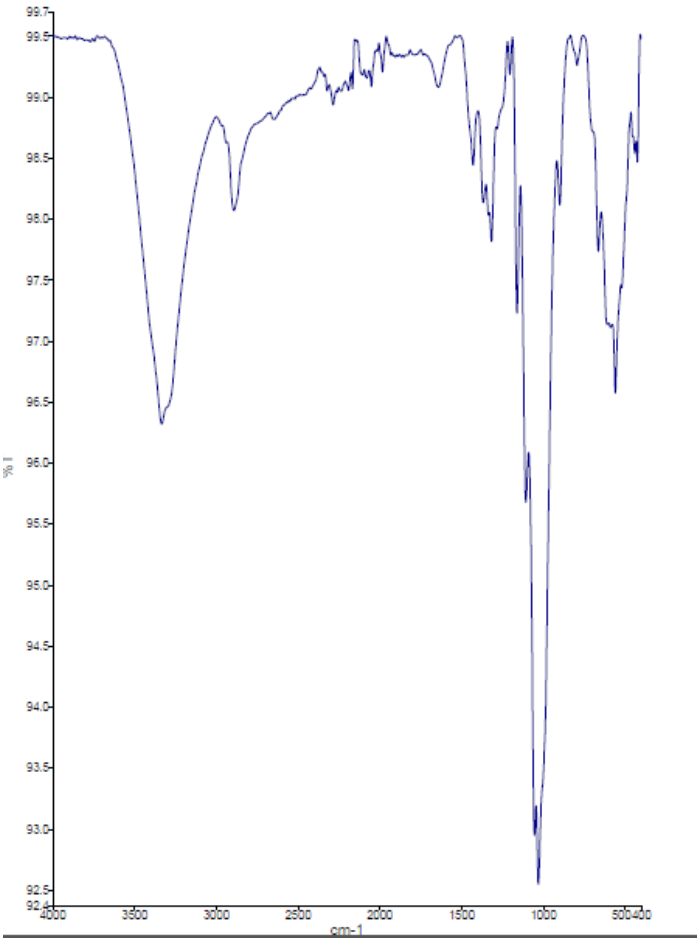


**Figure 8:** FTIR spectra of *V. radiata*.

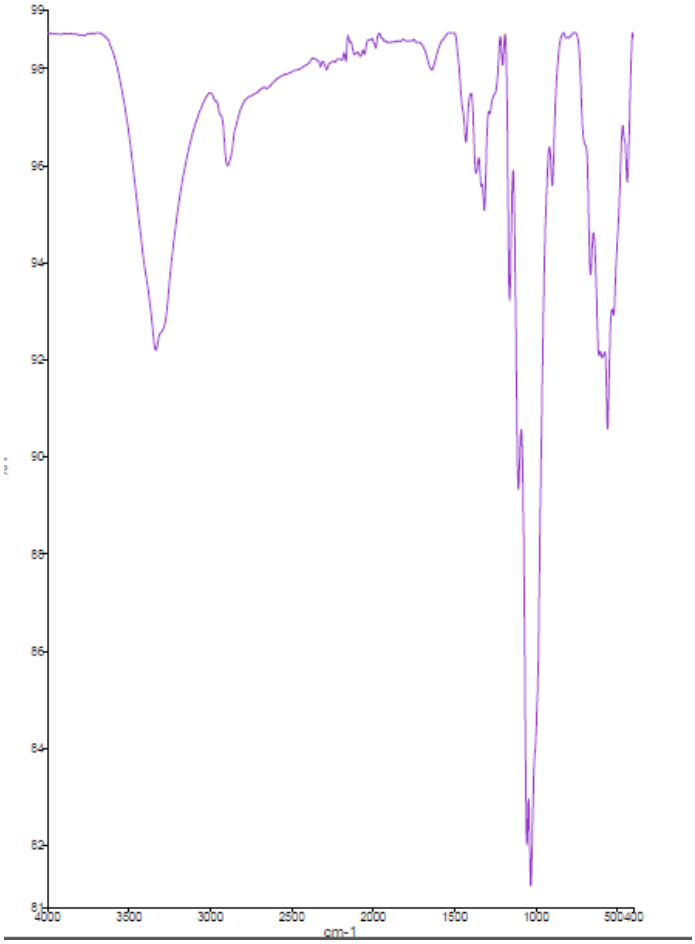


**Figure 9:** FTIR spectra of MCC.

depolymerized Braccoli fiber suggesting the relative slow release of the drug, Table 14.

The lipid accumulation study in 3T3 L1 preadipocytes showed that Metformin formulated in partially depolymerized Braccoli fiber showed greater effect when compared to other formulations and correlation between release time and high activity suggests the possible slow release of the drug. Other three formulations also however showed similar positive effect, Table 15.

The Metformin formulated in partially depolymerized Braccoli fiber resulted in significant increase in the glucose metabolism capability of INS-1 cells with or without glucose and the activity showed higher correlation with time suggesting slow release of the drug. The activity of the formulation in partially depolymerized Braccoli fiber was higher than the sustained release Metformin formulation. The formulation made with MCC showed least effect when compared to other three formulations Table 16.

Miglitol formulated with partially depolymerized Mung bean fiber (*Vigna radiata*) inhibited the enzyme at low concentration. Whereas, Miglitol formulated in partially depolymerized Braccoli fiber (B.o) showed activity only at higher concentration and also has taken longer time suggesting the possible poor release of the drug as in Table 17.

## DISCUSSION

Our present investigation has revealed that a value added, safe alternative to conventional excipient – MCC is possible from the most commonly used vegetable like *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean). The advantage of using such alternate depolymerized cellulose fiber is the additional nutritional value, safety and also such fiber being digestible.[21,22] Besides the above benefits, such fiber also would help in the treatment effectively by avoiding tachyphylaxis and reduced drug dosage if the same could delay the drug release.

The multifactorial disease like diabetes mellitus require lifelong medication, monitoring and lifestyle changes.[23] More often, the drug dosage required to bring down high blood glucose burden is also quite high where the scope for drug induced tachyphylaxis and the excipient led toxic effect, both are quite possible. Besides all the above, prolonged high dosage of metformin is also known to deplete $B_{12}$, an essential vitamin for our body function besides many other side effects.[24]

Our experiment has shown that metformin binds well with the fiber of *Brassica oleracea* (Broccoli) than with *Vigna radiata* (Mung bean) fiber. As a result of such firm binding, the release of metformin was observed to be relatively slow. However, such

**Table 4:** Ash values of *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) Sprouting and MCC.

| Sl. No | Herb | Ash value (% w/w) |
|---|---|---|
| 1 | *Brassica oleracea* (Broccoli) | 0.02 |
| 2 | *Vigna radiata* (Mung bean) sprouting | 0.05 |
| 3 | MCC | 0.17 |

**Table 5:** Bulk density, tap density and Hausner's ratio of *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) Sprouting and MCC.

| Parameters | Partially depolymerized *Brassica oleracea* (Broccoli) fibers | *Vigna radiata* (Mung bean) Sprouting | MCC |
|---|---|---|---|
| Bulk density (g/cm$^3$) | 0.41 | 0.425 | 0.29 |
| Tapped density (g/cm$^3$) | 0.47 | 0.48 | 0.34 |
| Hausner's ratio | 1.1 | 1.12 | 1.17 |

**Table 6:** Angle of repose of *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) Sprouting and MCC.

| Sl. No | Herb | Angle of repose(degrees) |
|---|---|---|
| 1 | *Brassica oleracea* (Broccoli) fiber | 51.1 |
| 2 | *Vigna radiata* (Mung bean) sprouting fiber | 50.9 |
| 3 | MCC | 49.7 |

**Table 7:** Tablet formulation with MCC, *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) Sprouting.

| Details | Proportion in mg | Formulation details | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| MCC | 50 | + | + | - | - | - | - |
| B.o fiber | 50 | - | - | + | + | - | - |
| V.r fiber | 50 | - | - | - | | + | + |
| Metformin | 500 | + | - | + | - | + | - |
| Miglitol | 20 | - | + | - | + | | + |

**Table 8:** Surface finish, Capping, Hardness, friability and disintegration test for formulations made of MCC, *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) Sprouting.

| Sl. No. | Parameters | Formulation details | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | Surface finish | S | s | s | s | s | S |
| 2 | Capping | Nil | Nil | Nil | Nil | Nil | Nil |
| 3 | Hardness (N) | 4 | 4.2 | 4.5 | 4.5 | 4.7 | 4.3 |
| 4 | Friability (%) | 1 | 0.8 | 0.7 | 0.7 | 08 | 0.7 |
| 5 | Disintegration (min) | 6.3 | 6.0 | 6.3 | 6.7 | 6.5 | 6.6 |

S= smooth

**Table 9:** Cytotoxic effect of test samples on SK N SH human neuroblastoma cells.

| Test samples | % Of cell death/concentration in µL | | |
|---|---|---|---|
| | 10 | 50 | 100 |
| Sustained release Metformin (500 mg) | 11 | 15 | 20 |
| Metformin in B.o (500 mg) | 8 | 10 | 13 |
| Metformin in *Vigna radiata* (500 mg) | 10 | 12 | 15 |
| Metformin in MCC (500 mg) | 9 | 14 | 16 |

**Table 10:** Neuroprotection effect of test samples against $H_2O_2$ induced damage.

| Test samples | % Of viable cells/concentration in µL | | |
|---|---|---|---|
| | 10 | 50 | 100 |
| Sustained release Metformin (500 mg) | 44 | 38 | 23 |
| Metformin in B.o (500 mg) | 38 | 50 | 63 |
| Metformin in *Vigna radiata* (500 mg) | 19 | 32 | 35 |
| Metformin in MCC (500 mg) | 11 | 16 | 26 |
| $H_2O_2$ – 1.0 Mm Concentration | 12 | | |

**Table 11:** Cytotoxic effect of test samples on kidney cells HK2.

| Test samples | % Of cell death/concentration in µL | | |
|---|---|---|---|
| | 10 | 50 | 100 |
| Sustained release Metformin (500 mg) | 28 | 18 | 17 |
| Metformin in B.o (500 mg) | 18 | 9 | 9 |
| Metformin in *Vigna radiata* (500 mg) | 25 | 16 | 11 |
| Metformin in MCC (500 mg) | 39 | 24 | 21 |

**Table 12:** TG accumulation in HepG2 cells.

| Test samples | Cellular TG in µg/mg protein vis-à-vis release time of test samples (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| NC | 30 | | | | | | | |
| HGHin | 62 | | | | | | | |
| Met B.o | - | 24 | 25 | 20 | 11 | 9 | 8 | 6 |
| Met V.r | - | 11 | 25 | 22 | 23 | 25 | 23 | 28 |
| Met.MCC | - | 18 | 27 | 29 | 31 | 29 | 30 | 31 |
| SR Met | - | 21 | 14 | 11 | 17 | 11 | 10 | 11 |

**Table 13:** Glucose utilization assay by HepG2 cells.

| Test samples | Glucose utilization (% control) vis-à-vis release time of test samples (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| Control | 100 | | | | | | | |
| 1,1-Dimethylbiguanide hydrochloride (0.1 µg/mL) | 183 | | | | | | | |
| Met B.o | - | 38 | 42 | 44 | 61 | 77 | 78 | 91 |
| Met V.r | - | 54 | 59 | 77 | 81 | 81 | 81 | 84 |
| Met.MCC | - | 56 | 57 | 60 | 78 | 77 | 80 | 81 |
| SR Met | - | 28 | 32 | 34 | 54 | 65 | 76 | 78 |

**Table 14:** Glucose utilization by L6 Myoblasts.

| Test samples | Glucose utilization (% control) vis-à-vis release time of test samples (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| Control | 100 | | | | | | | |
| Insulin (6µg/mL) | 165 | | | | | | | |
| Met B.o | - | 44 | 67 | 77 | 87 | 89 | 90 | 109 |
| Met V.r | - | 44 | 59 | 76 | 77 | 78 | 82 | 85 |
| Met.MCC | - | 68 | 77 | 78 | 77 | 78 | 81 | 83 |
| SR Met | - | 22 | 38 | 39 | 78 | 79 | 80 | 88 |

**Table 15:** Lipid accumulation in 3T3-L1 Preadipocytes.

| Test samples | Lipid accumulation (% control) vis-à-vis release time of test samples (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| Control | 100 | | | | | | | |
| Rosiglitazone (0.4 µg/mL) | 161 | | | | | | | |
| Met B.o | - | 67 | 88 | 91 | 92 | 98 | 101 | 107 |
| Met V.r | - | 64 | 69 | 79 | 88 | 91 | 93 | 96 |
| Met.MCC | - | 66 | 69 | 70 | 88 | 87 | 89 | 88 |
| SR Met | - | 67 | 76 | 79 | 84 | 85 | 88 | 90 |

**Table 16:** Glucose metabolism by INS-1 cells.

| Test samples | % viable cells vis-à-vis release time of test samples (hr) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 9 |
| Control w/ glucose | 28 | | | | | | | |
| Control w/o glucose | 11 | | | | | | | |
| Met B.o w/ | - | 33 | 39 | 45 | 52 | 78 | 79 | 82 |
| Met b.o w/o | - | 22 | 28 | 31 | 38 | 43 | 44 | 55 |
| Met V.r w/ | - | 61 | 79 | 72 | 80 | 81 | 81 | 82 |
| Met V.r /w/o | - | 44 | 45 | 45 | 48 | 52 | 55 | 67 |
| Met.MCC w/ | - | 33 | 39 | 34 | 41 | 45 | 48 | 53 |
| Met. MCC w/o | - | 11 | 11 | 12 | 13 | 17 | 18 | 21 |
| SR Met w/ | - | 63 | 64 | 69 | 70 | 71 | 72 | 70 |
| SR Met w/o | - | 23 | 21 | 26 | 28 | 29 | 30 | 32 |

**Table 17:** µ-glucosidase assay.

| Test samples | $IC_{50}$ value (µg/mL) vis-à-vis release time of Miglitol (minutes) | | | |
|---|---|---|---|---|
| | 10 | 20 | 30 | 35 |
| Miglitol in *Vigna radiata* fiber | 13 | 18 | 20 | 22 |
| Miglitol in B.o fiber | 27 | 39 | 51 | 62 |

binding does not seem to affect the functional aspect of the drug. Further the formulation can be made just with the fiber without any other excipients such as diluent, flow enhancer etc. Slow-release metformin is known to help to reduce the daily drug dosage from 1000 mg to 850. But such formulation has been made with conventional excipient Microcrystalline Cellulose (MCC).

We have performed a series of experiments with metformin formulation made with *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) fiber and the formulation with MCC. The sustained release, uncoated metformin tablet available in the market was used for comparison.

Our initial evaluation on the safety of both the fiber yielded positive result with the reference to neuroblastoma cells and kidney cells. The safety profile of both *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) are known for ages but for the want of scientific proof, we performed cytotoxic study with the above fiber.

Our research work on how the metformin from the excipients such as *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) would exhibit the therapeutic effect in comparison with the drug from MCC and sustained release tablet. For this purpose, we subjected the drug to passive dissolution in phosphate butter at different time intervals such as 1,2,3,4,5, 7 and 9 hrs and then a fixed quantity of sample from all the above experiment was tested on various cell lines. Initially we studied the cellular accumulation of TG in Hep G2 cells showed that the metformin from *Brassica oleracea* (Broccoli) fiber than *Vigna radiata* (Mung bean) fiber affected significantly the TG accumulation. The effective release time of metformin from the above formulation was 4 hrs whereas the metformin got released much earlier from sustained release tablet. So was with the formulation made with MCC. Our above findings led us to two possible affirmations such as

### Metformin may be binding firmly with *Brassica oleracea* (Broccoli) fiber

#### *The release of metformin from such fiber is near total*

We went further to confirm the above findings through a series of experiments using various cell lines. The glucose utilization pattern of HepG2 and L6 Myoblst cells post exposure to the test samples showed that metformin from *Brassica oleracea* (Broccoli) fiber significantly increased glucose utilization ability of the cells with late time release. The lipid accumulation in 3T3 preadiposites and glucose metabolism ability of INS 1 cells also yielded same result.

On the contrary, the depolymerized fiber of *Vigna radiata* (Mung bean) was not having any such selective binding preference for metformin and instead miglitol got released faster from the above excipient.

For treating type 2 diabetes mellitus, the combination of metformin and miglitol or vildagliptin are given where the later drug would hinder the surge and rush of glucose into blood post meal by hindering glucose conversion from carbohydrate. The above combination of drugs when administered, the release time of both drugs needs to be adjusted or timed through formulation engineering otherwise the alpha reductase inhibitor miglitol by delaying the glucose surge into blood stream would provide no benefit as the release and excretion of metformin would have happened prior to the above event. If metformin release is delayed, then the delayed import of glucose will be well metabolized by the cells where the timing of metformin empowering the cells would perfectly sync with glucose surge.[25]

The depolymerized fiber from *Brassica oleracea* (Broccoli) and *Vigna radiata* (Mung bean) met all most all characteristics of MCC with reference to physical and tablet formulating behaviour. With reference to colour, odour, taste, starch content, ash content, bulk density, tap density, angle of repose, all respect, the fiber could match MCC. The tablet made using the above fiber also showed high compressibility, smooth finish over the surface of the tablet, hardness, friability and disintegration time.

The alpha glucosidase inhibitor from *Vigna radiata* (Mung bean) fiber showed faster release whereas the metformin showed very slow but complete release from *Brassica oleracea* (Broccoli) fiber, complementing the treatment requirement of type 2 diabetes mellitus, perfectly.

Relatively poor response that we obtained from sustained release tablet of metformin may be due to the presence of several other excipients in the formulation. We, in the present study used the fiber or MCC without the addition of any other excipient. Therefore, the result obtained from sustained release tablet of metformin may be due to poor release or due to interference of other excipient in cell culture system, we could not elucidate clearly. All we know is that cell culture system is quite sensitive to artefacts.[26]

Findings of our present investigation may be just a beginning, but nevertheless it gives elaborate scope and confidence for further research to develop a value-added excipient, especially to reduce excipient accumulation led toxic side effects, reduce the dosage of the drug and to avoid tachyphylaxis due to prolonged drug exposure.[1]

## CONCLUSION

The micro cellulose fibers from *Brassica oleracea* and V*igna radiata* are the best alternatives for conventional cellulose fibers. The fibers isolated from *Brassica oleracea* showed slow release of metformin thus reduces drug dosage, tachyphylaxis. The release of Miglitol from *Vigna radiata* fibers was rapid and may compliment metformin therapy.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**MCC:** Micro crystalline cellulose; **NaOH:** Sodium hydroxide; **$H_2O_2$:** Hydrogen peroxide; **HCl:** Hydrogen chloride; **FBS:** Fetal bovine serum; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; **PBS:** Phosphate buffered saline; **TG:** Triglyceride; **$CO_2$:** Corbon dioxide; **DMEM:** Dulbecco's Modified Eagle Medium; **RPMI:** Roswell Park Memorial Institute Medium; **BSA:** Bovine Serum Albumin; **FCS:** Fetal calf serum; **DMSO:** Dimethyl Sulfoxide; **B.o:** *Brassica oleracea;* **V.r:** *Vigna radiata.*

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