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# COMPARATIVE CYTOTOXICITY OF *Curcuma longa* AND *Elettaria cardamomum* EXTRACTS CONJUGATED WITH GREEN-SYNTHESIZED MGO NANOPARTICLES ON PBMCS: ENHANCED EFFICACY OF *Elettaria cardamomum*

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**ABSTRACT**

This study explores the efficacy of combination herbs like Turmeric (*Curcuma longa*) and Pippali (*Piper longa*) (Curc+pip), Cardamom (*Elettaria cardamomum*) and Pippali (Card+pip) conjugated with green synthesized MgO nanoparticles (MgONPs) as a novel treatment modality for cardiovascular diseases in the context of diabetes mellitus. The cytotoxicity of extracts conjugated with green-synthesized MgONPs was investigated. Extracts were synthesized using the Soxhlet method and further characterized by HPTLC. Curry leaves were used to green synthesize MgONPs and characterized by DLS and FTIR. Cytotoxicity of herbal concoction was assessed using the MTT assay on Peripheral Blood mononuclear cells, of Normoglycemic and Type 2 Diabetes Mellitus individuals. HPTLC results revealed the presence of 3-butylligusticumlactone, 16-hydroxymedicagenic acid, and Curcumin in Turmeric. Cardamom showed a strong presence of terpenoids and Isopiperine and piperine in Pippali. MgONPs (55nm) demonstrated fair stability. Volume-dependent MTT assay using Cur+Pip and Card+Pip extract with and without NPs results exhibited low cytotoxicity on both NG and T2DM participants. The combination of Cur+Pip with and without MgONPs at 5µL presented viability up to to73.07%±0.03, In the case of 75µL, it declined up to 7.34%±0.21 in 24 hrs. Similarly, Card+Pip, exhibited 92.3%±1.1 viability at 5µL post 24 hrs and in 48 hrs, dropped to 70.15%±2.02. The Differences in mean viabilities were found to be non-significant in the case of treatments with and without NPs, hence well tolerated by cells. The combination with minimal cytotoxicity may exhibit promising cardioprotective effects as an alternative therapy. *Elettaria cardamomum* emerges as a Promising Candidate.

**Keywords:** Cardiovascular Diseases, Dynamic Light Scattering, High-Performance Thin Layer Chromatography, Fourier Transform Infrared Spectroscopy, Cytotoxicity, Nanoparticles.



## INTRODUCTION

Herbal medicines show therapeutic potential for various ailments, including immune support, due to their safety, efficacy, and affordability. Many rely on these remedies for primary healthcare. Diabetes mellitus (DM), affecting over 400 million people globally, leads to complications like neuropathy, nephropathy, and cardiovascular issues. Persistent high blood sugar leads to the formation of advanced glycation end products (AGEs). They are triggering inflammation and tissue damage. Herbal extracts have shown promise in managing diabetes through multiple mechanisms.[1] Curcumin, (*Curcuma longa*) a yellow polyphenol from turmeric, is known to reduce inflammation and has antioxidant properties. It has been studied as a treatment for various ailments. However, clinical trials have yet to confirm its therapeutic benefits despite extensive in-vitro and in-vivo research.[2] Cardamom (*Elettaria cardamomum*), a common household spice, can obliterate the formation of advanced glycation end products (AGEs). Its anti-inflammatory, and blood


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pressure-lowering effects, due to essential oils like terpenes and phenolic compounds, may benefit individuals with endothelial dysfunction in type 2 diabetes.[3,4]

Piperine was combined here for the bioavailability factor. Nanoparticles (NPs) are gaining attention in fields like materials science, energy, health, and biotechnology.[5] Green synthesis offers an eco-friendly, and biocompatible substitute to traditional methods, promoting sustainable growth. This makes it ideal for applications in energy storage, environmental remediation, medicine, and catalysis.[6,7,8,9,10] Peripheral blood mononuclear cells (PBMCs) are vital in immunological research, providing insights into systemic immune responses.[11,12] In type 2 diabetes mellitus (T2DM), alterations in PBMCs suggest a connection between the disease and immune system dysregulation.

Cytotoxicity testing aids pharmacological screening and quality control before in-depth mechanistic studies. PBMCs can assess the cytotoxic effects of nanoparticles, like MgONPs, on immune cells. The MTT assay is frequently used to assess cell viability and mitochondrial function, relying on the conversion of MTT into formazan crystals by living cells.[13,14] The objectives of the current study were: (i) To prepare and characterize hydroethanolic extracts, (ii) To perform green synthesis of MgONPs and assess their particle size and stability, and (iii) To combine the extracts of turmeric and pippali (Cur+Pip), as well as cardamom and pippali (Card+Pip), as herbal concoctions for cytotoxicity analysis on PBMCs, both with and without nanoparticles. Pharmacological treatments for type 2 diabetes pose several challenges, including the risk of vitamin B12 deficiency, hypoglycemia, and increased susceptibility to cardiovascular disease.[15] In contrast, herbal extracts conjugated with magnesium oxide nanoparticles (MgONPs) offer promising therapeutic potential. However, for these composites to be considered for future research and human application, they must demonstrate minimal toxicological risks. Therefore, this study underscores the necessity of cytotoxicity assays for such combinations to enable further exploration, particularly in addressing diabetes-related complications like cardiovascular diseases.

## EXPERIMENTAL

### Materials

*Curcuma longa, Elettaria cardamomum and Piper longum*, curry leaves *(Murraya koenigii),* Histopaque® (Himedia, density gradient 1.077 g/ mL), Dulbecco′s Phosphate Buffered Saline (Himedia), RPMI 1640 (Himedia, With L-Glutamine, 25mM HEPES buffer and Sodium bicarbonate), antibiotic– antimycotic (Penicillin and streptomycin Himedia), Fetal Bovine Serum (Himedia), MTT kit (ABCAM US), Distilled water,96 well plate (non-adherent), Trypan blue dye.

**Instruments**- ERBA EM360 Fully Automated clinical chemistry analyzer, Alere Affinion AS100 Analyzer, Soxhlet apparatus, CAMAG Linomat 5 andCAMAG TLC scanner (HPTLC), Malvern Zetasizer Lab blue, Synergy HTX Multimode Reader, BioTek, CO2 incubator.

### Herbal Extraction

### Turmeric, Cardamom and Pippali

Fresh green cardamom, turmeric rhizomes, and Pippali (25g each) were triturated and placed in a Soxhlet apparatus for extraction. A hydro-ethanol solvent (6:4 ratio of water to alcohol) was used, and the process took approximately 4 hours and 30 minutes. After extraction and removal of the solvent, the extract was sent for HPTLC (High-Performance Thin Layer Chromatography) analysis. The extraction yield was calculated by using this formula:

$$\text{Extraction Yeild (wt.\%)} = (\text{mass of extract} / \text{mass of raw material}) \times 100$$

### HPTLC analysis of *Elettaria cardamom and Piper longum* hydroethanolic extract of the whole plant

The test solution of herbal extracts from E. cardamom and P. longum was dissolved in 100 ml of ethanol and filtered. A 10.0 x 10.0 cm precoated silica gel 60 F 254 HPTLC plate (E. Merck KGaA) was used without pre-washing or alteration. The sample was applied in bands using a CAMAG Linomat applicator with a 100 µl syringe. The plate was then placed in an automated development chamber with a mobile phase of acetic acid, toluene, and ethyl acetate (7:2:1 v/v/v).

**Fourier Transform Infrared (FTIR) Spectroscopy**
FTIR was used to examine the surface functional groups of MgONPs in the infrared range 400 to 4000 cm 1 by the machine, Bruker ALPHA II FTIR Spectrometer.

**Green Synthesis of Nanoparticles**
Twenty-five grams of chopped leaves were boiled in 100 mL of distilled water for 10 minutes at 80–90°C to obtain an aqueous plant extract. Separately, 10 g of $MgSO_4$ was solubilized in 50 mL of deionized water on a hotplate, adjusting the temperature to 60–70°C. The leaf extracts were added dropwise until a yellow-brown ring formed at the junction, resulting in a nanosuspension. After evaporating the nanosuspension, the nanoparticles were sent for zeta sizer and FTIR analysis.

**Sample Preparation**
The trial solutions were prepared based on the established alkaloid percentages in herboceuticals. The samples were mixed in a ratio of 1:1.5 for turmeric and long pepper, and 1:3 for cardamom and Pippali, achieving a working concentration of 5 mg/ml. Additionally, 5 mg/ml of MgO nanoparticles (NPs) were dissolved in the same working volume as 5 mg/ml of Cur + Pippali and Cardamom + Pippali, with the final stock adjusted to 10 mg/ml.

**Isolation of PBMCs**
4 ml of human venous blood sample was taken and placed in citrate vials. The samples were mixed thoroughly and an equal amount of DPBS was added to the blood. The blood was gently layered on the top of the Ficoll Histopaque. The tubes were centrifuged for 30 minutes at 400 x g in a swing-out bucket (REMI). PBMCs formed in the interphase between Histopaque and medium were removed carefully followed by washing (centrifuge in 400 x g for 10 min) twice with 10 ml of DPBS. In the RPMI-1640 medium, the cells were again suspended. Using Trypan blue dye, cell viability was assessed. There was more than 97% cell viability.

**MTT Assay**
The cells were seeded in a non-adherent 96-well flat-bottom microtiter plate at a density of $5 \times 10^3$ cells/well. They were treated with *Car+Pip* and *Cur+Pip* in volumes of 5 µl, 15 µl, 25 µl, 50 µl and75 µl conjugated with and without NPs for 24 and 48 hours at 37°C in a CO2 incubator. 5 mg/mL MTT was added to each well and the plate was incubated for 4 hours at 37°C in a CO2 incubator. 120 µl of solvent (*provided in the kit containing 10% SDS in 0.001 N HCl acid*) was added to dissolve crystals. The plate was quantified using the Synergy HTX multimode microplate reader from BioTek at 540 nm. The relative cell viability (%) in the sample-treated wells with respect to the control wells was estimated by:

$$\% \text{ Cell Viability} = \frac{(\text{O.D of Test} - \text{O.D of Blank1}) \times 100}{(\text{O.D of Control} - \text{O.D of Blank2})}$$

Where Blank1 = Media + Treatment at various volumes such as 5 µl, 15 µl, 25 µl, 50 µl, and 75 µl in separate wells, Blank2 = Media alone

## RESULTS AND DISCUSSION

Medicinal plants have been central to traditional medicine, with emerging therapies offering promise in reducing cardiovascular risks for diabetics. In this study, Turmeric, Cardamom, and Pippali extracts were prepared using Soxhlet extraction and characterized by HPTLC. MgONPs were green-synthesized using curry leaves and characterized by DLS-based ZP and FTIR. Pippali was added in a fixed ratio to enhance the bioavailability of turmeric and cardamom. MgONPs were conjugated with the herbs and tested for cytotoxicity using the MTT assay. The cytotoxicity on PBMCs by treatment with two different compositions was made viz, *Cur+Pip* and *Card+Pip* with and without NPs to decide the safer dose for future research in CVDs.

**Biochemical Analysis**
The study consists of 10 participants including 5 normoglycemic and 5 T2DM patients satisfying the inclusion and exclusion criteria. The demographic information such as age, gender, and duration (*in*

*the case of T2DM*) was taken from participants and the results of FBS and HBA1C are mentioned in Tables-1 and 2.

Table-1: Demographic data of Normoglycemic and Diabetic subjects

| | (N=10) | | Statistics t Value | P Value |
|---|---|---|---|---|
| | NG (n=5) | T2DM(n=5) | | |
| FBS MG/DL (MEAN ± SD) | 82.6±8.02 | 167.4±37.57 | -4.84 | 0.0012** |
| (HBA1C%) (MEAN ± SD) | 4.74±0.27 | 9.97±0.89 | -12.7 | 0.00003** |
| AGE (MEAN ± SD) | 40±16.15 | 45.84±11.76 | -0.653 | 0.5*** |

Table-2: Comparison Between the Mean Values of Parameters in NG and T2DM Groups

| Normoglycemic | | | | | | |
|---|---|---|---|---|---|---|
| S. No. | Healthy Volunteer | Age | Gender | Duration of T2DM | FBS (mg/dl) | HbA1C (%) |
| 1 | Healthy Volunteer 1 | 25 | Female | No | 102 | 4.28 |
| 2 | Healthy Volunteer 2 | 37 | Male | No | 75 | 5.02 |
| 3 | Healthy Volunteer 3 | 22 | Male | No | 80 | 4.75 |
| 4 | Healthy Volunteer 4 | 51 | Male | No | 79 | 4.65 |
| 5 | Healthy Volunteer 5 | 65 | Female | No | 77 | 5.02 |
| T2DM | | | | | | |
| S. No. | Patient | Age | Gender | Duration of T2DM | FBS (mg/dl) | HbA1C (%) |
| 1 | Patient 1 | 45 | Female | 5 years | 132 | 9.84 |
| 2 | Patient 2 | 57 | Male | 7 years | 230 | 11.47 |
| 3 | Patient 3 | 26 | Male | 2 years | 170 | 9.06 |
| 4 | Patient 4 | 45 | Male | 6 years | 156 | 9.84 |
| 5 | Patient 5 | 52 | Female | 4 years | 149 | 9.65 |

Count of FBS, HbA1C, and Age associated with NG/T2DM. N = Total Count; n = Individual counts, 2 sampled independent t-tests were performed ($p<0.05$).
* Indicates nonsignificant values ($p>0.05$)
** Indicates significance level up to 0.001
*** Indicates highly significant level >0.001

## HPTLC

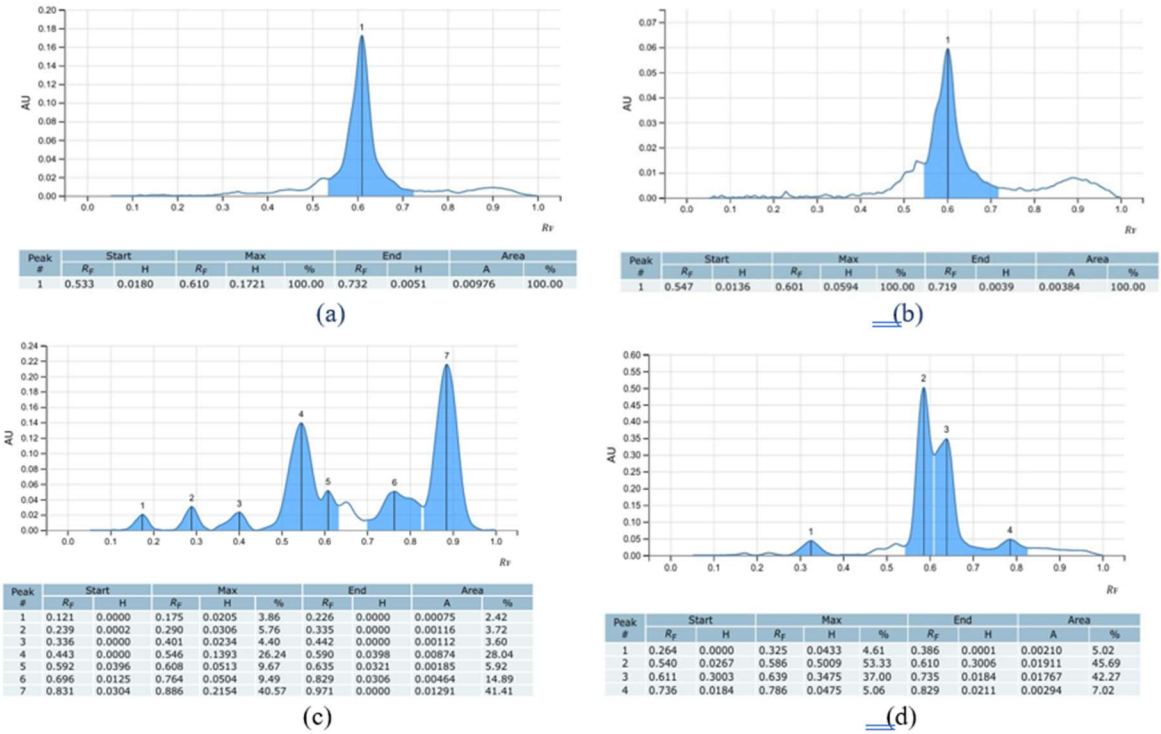
The phytochemical profile of the plant was established by determining the total number of peaks, peak heights, peak areas, percentage areas, and Rf values, as shown in the tables. (Fig.-1)

In case of Cardamom, graph showed that in hydroethanolic extract, single peak was observed with Rf value 0.533 and area 0.00976 with 100% saturation of phytochemical terpenoids including contents like Myrcene, Cineole, linalool, Beta caryophyllene etc when run at 10 µL with the concentration of 5 mg/ml. Similarly, other graph (also reflects 100% saturation of terpenoids with Rf value 0.547 and area 0.00384 at 5µL. (Fig.-1a and b) Cardamom contains compounds such as cineole and limonene, which have shown anti-inflammatory and antiplatelet effects.

In case of Pippali hydroethanolic extract, 4 peaks were observed but the major area was covered by isopiperine (45.69%) (Peak 2) with Rf, 0.540 and area as 0.01911 showing the saturation of 45.69%. On the other hand, peak 3 at Rf 0.611 was assigned as piperine (42.27%) with area 0.01767 in 10 µL. (Fig.-1c) Pippali and its active compound, piperine, act as bioenhancers by increasing the bioavailability and efficacy of various drugs and nutrients. This occurs by inhibiting metabolic enzymes, enhancing intestinal permeability, and blocking efflux pumps. Pippali is commonly combined with turmeric and cardamom in traditional medicine, as their medicinal properties work synergistically to boost overall therapeutic effectiveness.[16]Strong antioxidant curcumin has the ability to combat free radicals and lessen oxidative stress, which is a major contributor to cardiovascular diseases. [17]

The result for turmeric shows a rf value of 0.696 plotted in the area of 0.00465 with the % of 14.86 identified as curcuminoid similarly 0.443 in the plotted area of 0.00874 with% 28.04 is the flavonoid which is derived 16- hydroxy medicagenic acid and rf value of 0.831 in the area of 0.01291with the % 41.41 3-

butylligusticumlactone. Along with these identified phytochemicals, phenols, terpenoids, and glycosides exist, respectively. This data indicates presence of pure and quantifiable amount of curcuminoids (Fig.-1d).




| Peak # | Start $R_F$ | Start H | Max $R_F$ | Max H | Max % | End $R_F$ | End H | Area A | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.533 | 0.0180 | 0.610 | 0.1721 | 100.00 | 0.732 | 0.0051 | 0.00976 | 100.00 |

| Peak # | Start $R_F$ | Start H | Max $R_F$ | Max H | Max % | End $R_F$ | End H | Area A | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.547 | 0.0136 | 0.601 | 0.0594 | 100.00 | 0.719 | 0.0039 | 0.00384 | 100.00 |

| Peak # | Start $R_F$ | Start H | Max $R_F$ | Max H | Max % | End $R_F$ | End H | Area A | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.121 | 0.0000 | 0.175 | 0.0205 | 3.86 | 0.226 | 0.0000 | 0.00075 | 2.42 |
| 2 | 0.239 | 0.0002 | 0.290 | 0.0306 | 5.76 | 0.335 | 0.0000 | 0.00116 | 3.72 |
| 3 | 0.336 | 0.0000 | 0.401 | 0.0234 | 4.40 | 0.442 | 0.0000 | 0.00112 | 3.60 |
| 4 | 0.443 | 0.0000 | 0.546 | 0.1393 | 26.24 | 0.590 | 0.0398 | 0.00874 | 28.04 |
| 5 | 0.592 | 0.0396 | 0.608 | 0.0513 | 9.67 | 0.635 | 0.0321 | 0.00185 | 5.92 |
| 6 | 0.696 | 0.0125 | 0.764 | 0.0504 | 9.49 | 0.829 | 0.0306 | 0.00464 | 14.89 |
| 7 | 0.831 | 0.0304 | 0.886 | 0.2154 | 40.57 | 0.971 | 0.0000 | 0.01291 | 41.41 |

| Peak # | Start $R_F$ | Start H | Max $R_F$ | Max H | Max % | End $R_F$ | End H | Area A | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.264 | 0.0000 | 0.325 | 0.0433 | 4.61 | 0.386 | 0.0001 | 0.00210 | 5.02 |
| 2 | 0.540 | 0.0267 | 0.586 | 0.5009 | 53.33 | 0.610 | 0.3006 | 0.01911 | 45.69 |
| 3 | 0.611 | 0.3003 | 0.639 | 0.3475 | 37.00 | 0.735 | 0.0184 | 0.01767 | 42.27 |
| 4 | 0.736 | 0.0184 | 0.786 | 0.0475 | 5.06 | 0.829 | 0.0211 | 0.00294 | 7.02 |

Fig.-1: HPTLC data of Cardamom and Pipali extract, (a) HPTLC fingerprint chromatogram of 10µL hydroethanolic extract (5mg /mL) solution of *Elettaria cardamom,* (b) HPTLC fingerprint chromatogram of 5µL hydroethanolic extract (5mg /mL) solution of *Elettaria cardamom,* (c) HPTLC fingerprint chromatogram of 10µL hydroethanolic extract (5mg /mL) solution of *Piper longum, (d)* HPTLC fingerprint chromatogram of 7 µL hydroethanolic extract (5mg /mL) solution of *Curcuma longa.*

## Characterization of NPs

### Dynamic Light Scattering (DLS) and Zeta Potential (ZP)

Green synthesis of NPs often results in biocompatible and less toxic.[18] In this study, the use of curry leaves to synthesize MgONPs represents a green and innovative approach to enhance the medicinal activity of herbal formulations. When used as an adjuvant to turmeric and Pippali, MgONPs can significantly boost their therapeutic properties.[19]

Hence size obtained as 54.95 nm. ZP indicates stability of nanoparticles as it is a direct indicator of surface charge hence, found to be -0.87mV(Fig.-2), Table-3. Number of studies have proven that metal NPs in the size range of 10-100nm can effectively enter and interact with PBMCs.[20] Since they can successfully carry medications and have an increased permeability and retention effect, NPs with a diameter range of 10 to 100 nm are typically regarded as appropriate. Phagocytes are expected to remove larger-than-100 nm particles from the bloodstream.[21]

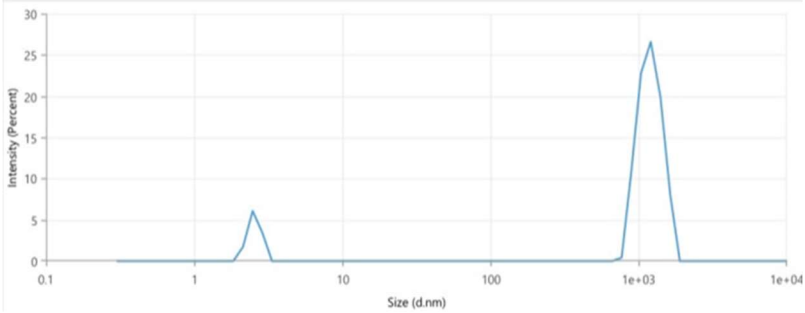



Fig.-2: Graphs of dynamic light scattering (DLS) and zeta potential analysis with particle size: 54.95 nm.

Table-3: Data of dynamic light scattering (DLS) and zeta potential analysis with particle size: (a) 54.95nm

| Name | Mean |
|---|---|
| Z- Average (nm) | 54.95 |
| Polydispersity Index (PI) | 0.6542 |
| Intercept | 0.6947 |
| Peak 1 Mean by Intensity ordered by area (nm) | 1207 |
| Peak 2 Mean by Intensity ordered by area (nm) | 2.552 |
| Peak 1 Area by Intensity ordered by area (%) | 88.76 |
| Peak 2 Area by Intensity ordered by area (%) | 11.24 |
| Derived Mean Count Rate (kcps) | 1.652 |
| Dv (10) | 1.963 |
| Dv (50) | 2.463 |
| Dv (90) | 3.112 |

**FTIR for NPs**

The MgO nanoparticle spectra and the analysis conducted in the 500–4000cm$^{-1}$ range are displayed in Fig.-3. The O-H bond vibrations of the hydroxy group were found to be in the strong infrared band around 3436 cm$^{-1.}$

The peak at 2330cm$^{-1}$ was observed for the C–N bond vibrations. The peak at 608 cm$^{-1}$ indicates the presence of MgONPs.[22,23] At the peak 1655 cm$^{-1}$ and 1015cm$^{-1}$ is related to C–N and C=O bond vibrations respectively. The range from 107-1145 represents C=O where peak obtained was 1145 cm$^{-1}$ and 1072cm$^{-1}$. (Fig.-3)

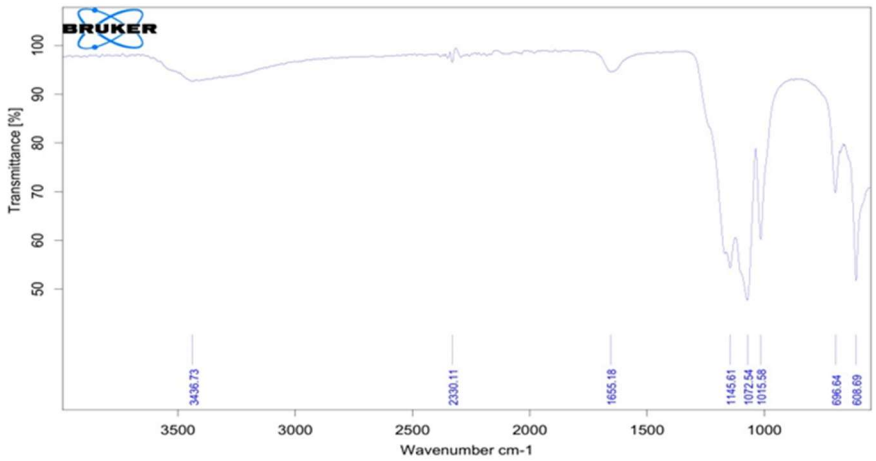



Fig.-3: FTIR spectrum of MgO nanoparticles

**PBMC viability after treatment with extracts**

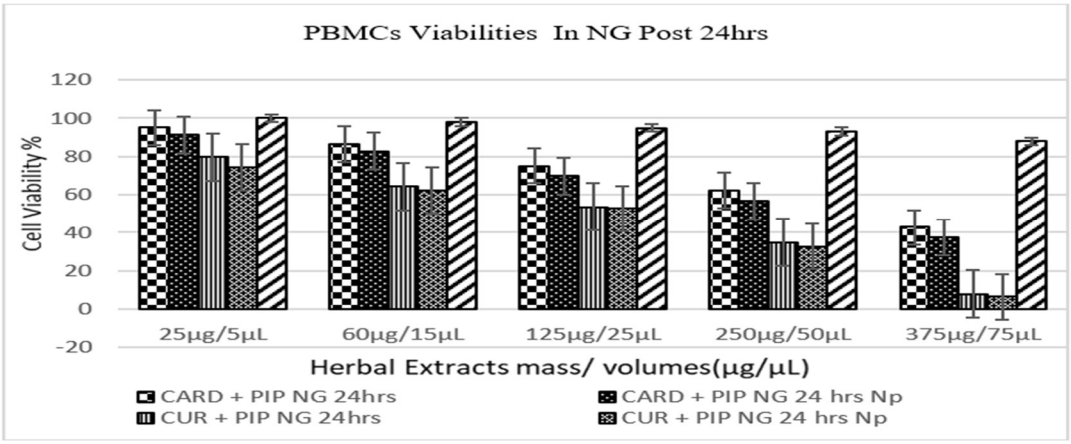
PBMC viability after treatment with *Cur+Pip, Card+Pip* and *Cur+Pip+Nps*, *Card+Pip+NPs* is shown in Fig.-4 in the case of NG and T2DM groups for 24 hrs. and 48 hrs. respectively. For MTT assay in all test, the untreated cells of NG and T2DM individuals were evaluated as the control. There was not much significant difference in the viability of PBMCs in the NG and T2DM groups especially when treated with their respective *NPs* whether it is 24 or 48hrs. ($p>0.05$) (Table-4).

In case of turmeric, results after 24 hrs of treatments indicated cell viability in the order of 73.99±1.97, 62.85±0.60, 51.71±1.22, 33.18±0.67 and 6.69±0.54 for the concentrations 25µg/5µL, 60µg/15µL, 125µg/25µL, 250µg/50µ and 375µg/75µL respectively, where data is represented as mean values ± SEM of both NG and T2DM including with and without NPs in 24 hrs. In 48 hrs., the results were 45.83±1.28, 36.11±0.90, 24.96±1.08, and 4.19%±0.20 respectively for the above-mentioned concentrations.

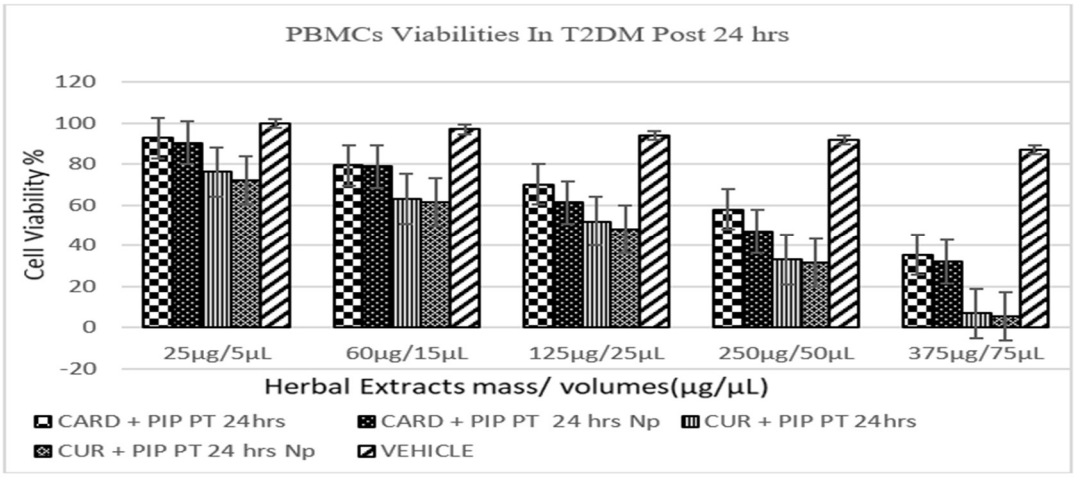
In case of Cardamom, results after 24 hrs. of treatments indicated cell viability in the order of 92.41%±0.6, 81.94±1.77, 69.16 ±2.88, 52.67±2.76 and 37.00±2.21 for the concentrations mentioned above respectively.

Hence it is evident that cardamom rendered more viability at the same concentration as compared to turmeric (Fig.-5a).

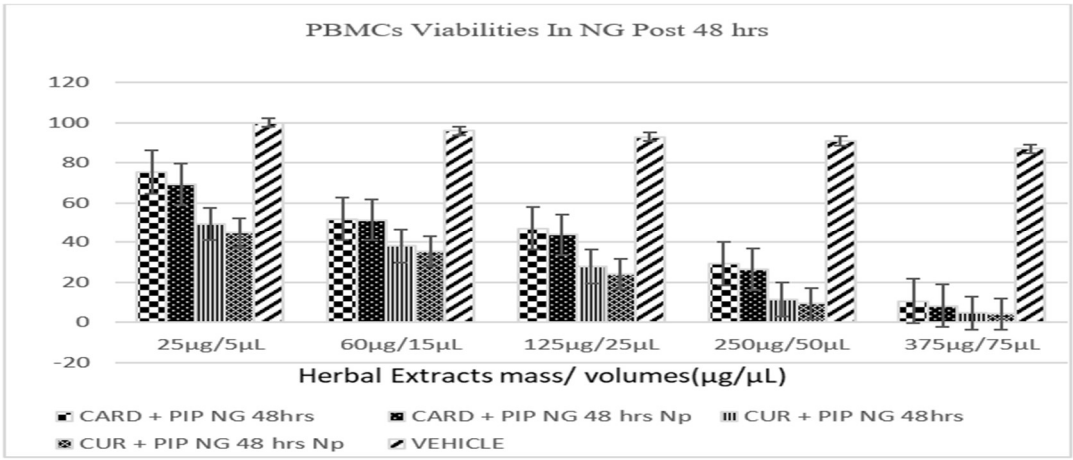
In 48 hrs., results were 71.9%±1.74, 54.56±1.80, 42.09±1.79, 27.57±1.05 and 9.17%±0.59 respectively. Hence, we can easily predict the drastic variations in these two treatments in 24 and 48 hrs. ($p<0.05$), Fig.-5b.



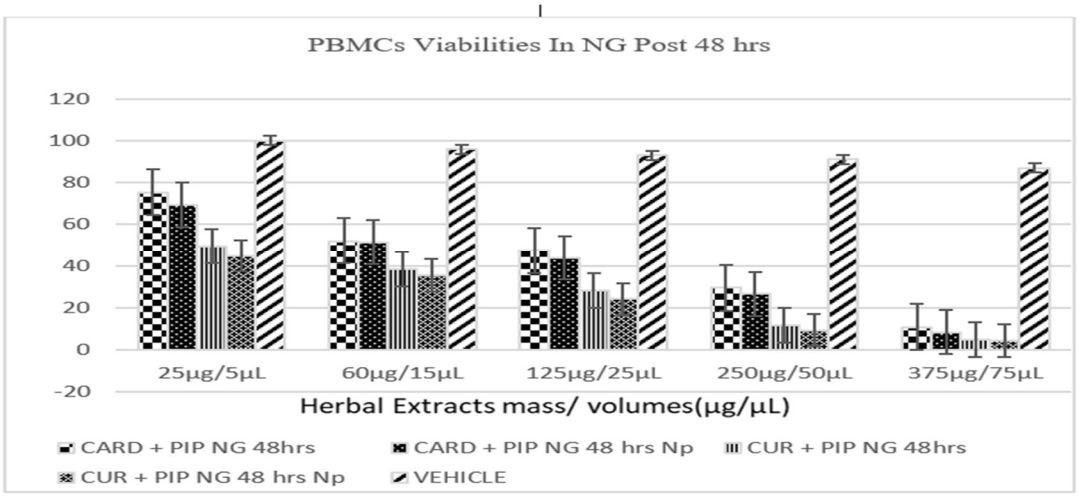

(a)




(b)

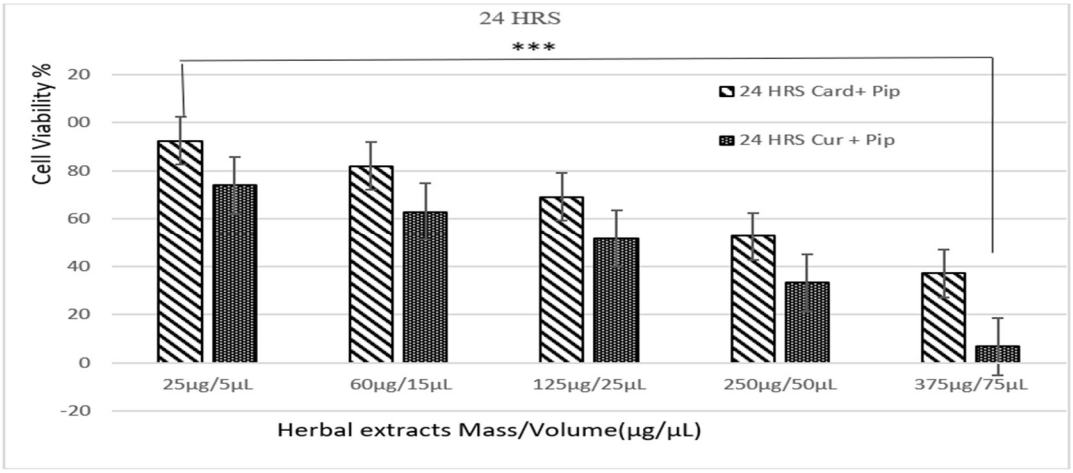



(c)

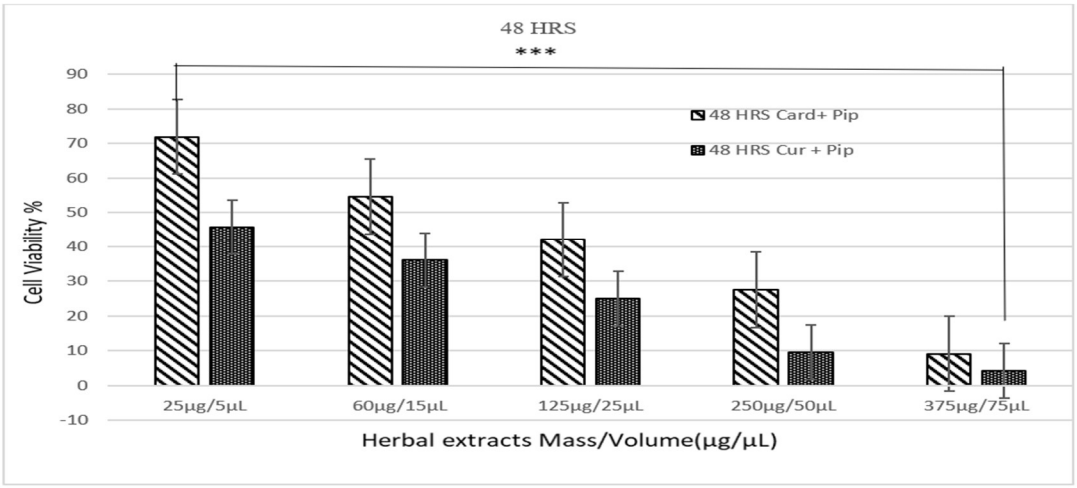



(d)

Fig.-4 Comparison of viability of PBMCs post-treatment 24 and 48 hrs.




(a), Post 24Hrs treatment




(b), Post 48 Hrs treatment

Fig.-5: Viability of PBMCs post treatment of Cardomom and Turmeric

Respective bar represents the cumulative mean values of viabilities with and without NPs as well as NG and T2DM at particular volumes in given time.

The vehicle (hydro ethanol) showed minimal effect on PBMCs viability($p<0.05$), as it was found to be <90% at all the volumes (5µL-75µL). It is noteworthy, that there is a significant difference between the cell viabilities in 24 and 48 hrs irrespective of NG and T2DM cases with and without NPs ($p<0.05$) in both the treatments i.e., *Cur+Pip* and *Card+Pip* (Table-4).

Cell viability was assessed using the MTT method in PBMCs from NG individuals and T2DM patients, exposed to different mass/volume with and without MgONPs. (a) Cytotoxicity of Card+Pip and Cur+Pip in NG individuals after 24 hours, with and without NPs. (b) Cytotoxicity of the same treatments in T2DM patients after 24 hours. (c) Cytotoxicity in NG individuals after 48 hours. (d) Cytotoxicity in T2DM patients after 48 hours. Viability is expressed as mean values.

Table-4**:** Comparison of p values of different volumes of treatments by using Wilcoxon sign ranked test

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG without NPs | 0.042 | 0.043 | 0.042 | 0.042 | 0.042 |
| 2. | NG with NPs | 0.039 | 0.043 | 0.042 | 0.042 | 0.041 |
| 3. | PT without NPs | 0.041 | 0.042 | 0.042 | 0.043 | 0.042 |
| 4. | PT with NPs | 0.039 | 0.043 | 0.042 | 0.043 | 0.042 |
| 5. | NG with vehicle | 0.191* | 0.149* | 0.191* | 0.149* | 0.149* |
| 6. | PT with vehicle | 0.191* | 0.149* | 0.149* | 0.149* | 0.149* |

**With vs Without NP**

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG 24 hrs | 0.034 | 0.078* | 0.043 | 0.43* | 0.42* |
| 2. | NG 48 hrs | 0.042 | 0.102* | 0.180 * | 0.042 | 0.066* |
| 3. | PT 24 hrs | 0.066* | 0.414* | 0.043 | 0.039 | 0.109* |
| 4. | PT 48 hrs | 0.042 | 0.042 | 0.039 | 0.066* | 0.077* |

**Turmeric (Curc+pip)**

(A) NG vs PT

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | 24 hrs without NPs | 0.078* | 0.102* | 0.104* | 0.109* | 0.129* |
| 2. | 24 hrs with NPs | 0.041 | 0.414* | 0.068* | 0.461* | 0.197* |
| 3. | 48 hrs without NPs | 0.078* | 0.216* | 0.042 | 0.496* | 0.180* |
| 4. | 48 hrs with NPs | 0.083* | 0.216 * | 0.786* | 0.042 | 0.458* |

(B) 24 vs 48

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG without NPs | 0.043 | 0.043 | 0.039 | 0.041 | 0.041 |
| 2. | NG with NPs | 0.042 | 0.043 | 0.039 | 0.043 | 0.102* |
| 3. | PT without NPs | 0.042 | 0.043 | 0.043 | 0.043 | 0.041 |
| 4. | PT with NPs | 0.042 | 0.039 | 0.498* | 0.042 | 0.066* |
| 5. | NG with vehicle | 0.191* | 0.149* | 0.191* | 0.149* | 0.149* |
| 6. | PT with vehicle | 0.191* | 0.149* | 0.149* | 0.149* | 0.149* |

(C) With vs Without NP

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG 24 hrs | 0.042 | 0.104* | 0.180* | 0.102* | 0.038 |
| 2. | NG 48 hrs | 0.068* | 0.066* | 0.043 | 0.068 * | 0.593* |
| 3. | PT 24 hrs | 0.042 | 0.102* | 0.042 | 0.102* | 0.059* |
| 4. | PT 48 hrs | 0.066* | 0.138* | 0.492* | 0.041 | 0.785* |

**Cardamom + Pippali and Curcuma +pippali**

(A) NG vs PT

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | 24 hrs without NPs | 0.028 | 0.008 | 0.008 | 0.011 | 0.009 |
| 2. | 24 hrs with NPs | 0.027 | 0.043 | 0.007 | 0.021 | 0.013 |
| 3. | 48 hrs without NPs | 0.019 | 0.239* | 0.012 | 0.496* | 0.066* |
| 4. | 48 hrs with NPs | 0.019 | 0.305* | 0.126* | 0.036 | 0.262* |

(B) 24 vs 48

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG without NPs | 0.033 | 0.007 | 0.005 | 0.005 | 0.005 |
| 2. | NG with NPs | 0.019 | 0.239* | 0.012 | 0.496* | 0.066* |
| 3. | PT without NPs | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |
| 4. | PT with NPs | 0.005 | 0.005 | 0.073* | 0.005 | 0.008 |
| 5. | NG with vehicle | 0.191* | 0.149* | 0.191* | 0.149* | 0.149* |
| 6.. | PT with vehicle | 0.191* | 0.149* | 0.149* | 0.149* | 0.149* |

**With vs Without NP**

| S. No. | Criteria | Treatment 1 (p-value) | Treatment 2 (p-value) | Treatment 3 (p-value) | Treatment 4 (p-value) | Treatment 5 (p-value) |
|---|---|---|---|---|---|---|
| 1. | NG 24 hrs | 0.005 | 0.016 | 0.018 | 0.012 | 0.005 |
| 2. | NG 48 hrs | 0.005 | 0.011 | 0.018 | 0.005 | 0.008 |
| 3. | PT 24 hrs | 0.007 | 0.057* | 0.005 | 0.011 | 0.016 |
| 4. | PT 48 hrs | 0.007 | 0.016 | 0.073 | 0.008 | 0.136* |

Where treatments are herbal extract mass/given volume. Treatment 1 = 25µg/5µL, treatment 2 = 60µg/15µL treatment 3 = 125µg/25µL, treatment 4 = 250µg/50µL, treatment 5 = 375µg/75µL.
*Indicates non-significant p-value($p<0.05$)

## CONCLUSION

This study is the first to evaluate the combination of turmeric, cardamom, and Pippali, enhanced by green-synthesized MgONPs, as an alternative medicine. The goal was to leverage the synergistic effects of the herbs and nanoparticles for improved therapeutic outcomes. Results showed no significance in PBMC viability between NG and T2DM groups treated with Cur+Pip+NPs or Card+Pip+NPs at 24 and 48 hours ($p>0.05$). However, Card+Pip treatments showed higher cell viability than Cur+Pip, with or without NPs, suggesting potential for future concentration-dependent studies or polyherbal combinations like Turmeric+Cardamom+Pippali.

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## CONFLICT OF INTERESTS

The Authors declare that there is no conflict of Interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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