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# Formulation and Evaluation of Gumghatti Nanoparticles Comprising of Cyclophosphamide For Enhanced Antitumor Activity

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## ABSTRACT

In this current research work the nanoparticles containing Cyclophosphamide using natural Gumghatti as a polymer has been formulated for the enhanced anticancer activity. The nanoprecipitation method has been utilized for the preparation of Cyclophosphamide comprising natural Gumghatti as polymer. The prepared nanoparticles have shown the average particle size, polydispersity index and zeta potential of 143.4 nm, 0.16 and 28.5 mV respectively. Further the encapsulation efficiency, percentage drug loading and percentage yield for all the formulations were substantial, especially for the trial 6 the values observed were 93.56, 83.55 and 76.54 respectively. The *invitro* release of Cyclophosphamide Gumghatti nanoformulations and were determined by dissolution tester by USP apparatus II in 900ml phosphate buffer pH 6.8. The dissolution media were maintained at 37±0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 15 mg at specified time intervals (5, 10 15, 20, 25 30 60, 90 and 120 minutes.) The results shows that prepared nanoparticles having 98.53 % of drug release in 120 minutes, further it indicates more than 95 % of drug release in 2 hours. *Invivo* anticancer activity has been performed using Swiss albino mice, the results indicated that the enhanced anticancer activity of the prepared nanoformulations.

**Keywords:** Cyclophosphamide, Gumghatti, Nanoprecipitation, *invitro* drug release, anticancer activity.

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## INTRODUCTION

In the last decades, the number of patients receiving chemotherapy has considerably increased. The development of reliable and novel formulations to treat the patients became necessary. From the discovery of new substances to patient administration for the panacea, as the pharmaceutical fields are concerned with the novel formulation of drugs with increased efficacy[1-6]

Cyclophosphamide is amongst the best and broadly used antineoplastic medications. Besides, it is likewise a strong immunosuppressive specialist and the most regularly utilized medication as a part of blood and marrow transplantation (BMT). It was at first incorporated to specifically target growth cells, despite the fact that the speculated system of tumor specificity (enactment by disease cell phosphamidases) happened to be unessential to its action. In any case, cyclophosphamide's novel digestion system and inactivation by aldehyde dehydrogenase is in charge of its unmistakable cytotoxic properties. Differential cell articulation of aldehyde dehydrogenase affects the anticancer remedial file and immunosuppressive properties of cyclophosphamide[7]. The novel formulation Cyclophosphamide has been prepared for better antitumor activity

Cyclophosphamide (Cytoxan; Cy) is an alkylating agent with cytotoxic and immunosuppressive activities. The parent compound is inactive in vitro and exerts its biologic activity through metabolites, mainly phosphoramide mustard generated by hepatic microsomal enzymes. The exact mode of cytotoxic and immunosuppressive action of Cyclophosphamide at cellular level is not completely understood. Myelosuppression, hemorrhagic cystitis, alopecia, and gonadal damage are the main toxic effects. The contemporary research proposes that nanotechnologies may prompt the advancement of novel malignancy treatment[9]. Nanoparticles with their interesting physical and synthetic properties hold extraordinary trusts in the improvement of warm based treatments against human malignancies. The promising approach of developing a nanoformulation with the novel polymer Gumghatti with the drug cyclophosphamide.[10-17]

## MATERIALS AND METHOD

Cyclophosphamide and gumghatti was purchased from Sigma aldrich, All the reagents and solvents used were analytical grade and standard.

**Formulation of Cyclophosphamide gumghatti Nanoparticles**

Cyclophosphamide was mixed with water to prepare solution and add (DMSO) as cosolvent to make the inner phase more homogeneous. Then 150mg of gum ghatti was dissolved in acetone, and the solution was added to the drug to form dispersion. The dispersion was add to 10 ml of aqueous ethanol solution (70%)after 5 minutes of mixing, the organic solvent was removed by

evaporation at 38°cunder normal pressure, nanoparticles were separated by using cooling centrifuge at 10000 rpm for 20 min supernatant was removed and nanoparticles was washed with water and dried at room temperature in a desicator.

**Encapsulation efficiency of Cyclophosphamide gumghatti Nanoparticles**

Encapsulation efficiency, which is the percentage of the actual amount of drug encapsulated in the polymeric carrier relative to the total amount of drug taken for Nanoparticles preparation, is calculate by using the following equation:

%Encapsulation Efficiency = (Actual drug loading/ Theoretical drug loading) × 100

To calculate actual drug loading an accurately weighed quantity of Cyclophosphamide was sonicate in 10 ml of methanol for 5 minutes and filter through 0.45 µl syringe filter. Cyclophosphamide concentration is analyzed by measuring the absorbance at 287 nm using UV–Vis spectrophotometer.

**Dissolution Study**

The dissolution profiles of Cyclophosphamide Gumghatti nanoformulations and were determined in a dissolution tester by USP apparatus II in 900ml phosphate buffer pH 6.8. The dissolution media were maintained at 37±0.5°C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 15 mg. At specified time intervals (5, 10 15, 20, 25 30 60, 90 and 120 minutes.) 5ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium at 37° C to maintain a constant total volume. Samples were filtered through a 0.22µm nylon membrane filter and assayed for drug content spectrophotometrically at 363 nm using UV-1700,Shimadzu Corporation, Japan UV/Vis double beam spectrophotometer after appropriate dilution with phosphate buffer pH 6.8.Cumulative percentage of drug dissolved in the preparations was calculated using calibration equations. Dissolution tests were performed in three vessels per formulation ($n$ = 3).

**Anticancer activity**

Anti cancer activity in mice after acclimatization, mature male Swiss albino mice divided into four groups (n=10) and given food and water ad libitum. All the groups were injected with DLA Cells (1×106 cells/mouse.i.p)and divided following groups are given below

G**roup I (n=6):** Control: Rats treated with 1%Carboxy Methyl Cellulose (CMC) Suspension.

**Group II (n=6):** Tumor induced rats treated with CMC

**Group III (n=6):** Sensitized rats treated with nanoparticles of cyclophosphamide 100 mg/kg/p.o.

**Group IV (n=6):** positive control: Rats treated with cyclophosphamide 100 mg/kg/p. o

The above drugs and solvents were administered orally and continued for 14 consecutive days. The dose of EVN was selected based on previous study on hepatoprotective activity.10 On day 15, five mice of each group were sacrificed 24 h after the last dose and the rest were kept with food and water ad libitum to check the increase in the life span of the tumor hosts. The effect of cyclophosphamide on tumor growth and host's survival time were examined by studying the parameters like tumor volume, tumor cell count, and increase in life span.

**Experimental protocol**:

Healthy Adult Swiss Albino mice were weighed and divided into five groups of six each. Group I acted as tumor control, Group II, III and IV received 50, 100, 150 mg/kg of extract and Group V standard drug Cyclophosphamide 80 mg/kg body weight. DLA cells were maintained as ascites tumors in Swiss albino mice. The cells were aspirated, washed thrice in normal saline counted using a haemocytometer and cell suspension of one million cells/ml was prepared. One ml of this suspension was injected intraperitoneally for 9 days. On the 10th day, body weight of the animal was noted. 24 hours after the last dose of the drug, one set of the animals were sacrificed and the weight of the vital organs such as spleen, thymus, liver and kidney were recorded and expressed as relative organ weights. Solid tumor volume: Tumor was induced by injecting DLA cells (1x106 cells/animal) subcutaneously to the right hind limb of the animals for five groups. The radii of the tumor were measured using Vernier Calipers at 5 days intervals for one month starting with 15th day. The volume of the tumor was calculated using the formula $V=4/3 \prod r1^2 r^2$ where 'r1' and 'r' represent the major and minor diameter respectively. This was compared with untreated[18-26].

## RESULTS AND DISCUSSION

**In-vitro Release Study Of Cyclophosphamide Nanoparticles**

**Calibration Curve**

| Concentration (µg/ml) | Absorbance (nm) |
|---|---|
| 20 | 0.117 |
| 40 | 0.251 |
| 60 | 0.39 |
| 80 | 0.518 |
| 100 | 0.636 |

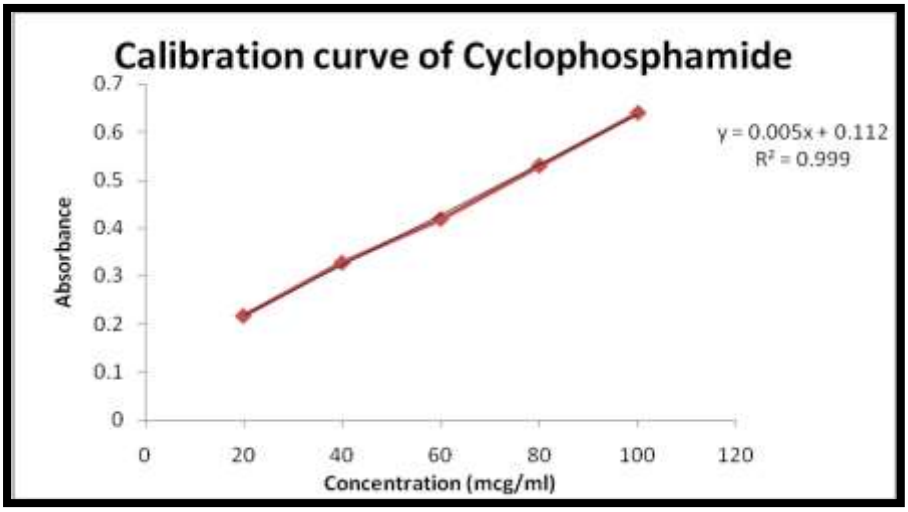


**Figure 2: Calibration curve of Cyclophosphamide in water**

**Calibration curve of Cyclophosphamide**

| Concentration (µg/ml) | Absorbance (nm) |
|---|---|
| 0 | 0 |
| 20 | 0.117 |
| 40 | 0.251 |
| 60 | 0.409 |
| 80 | 0.518 |
| 100 | 0.636 |

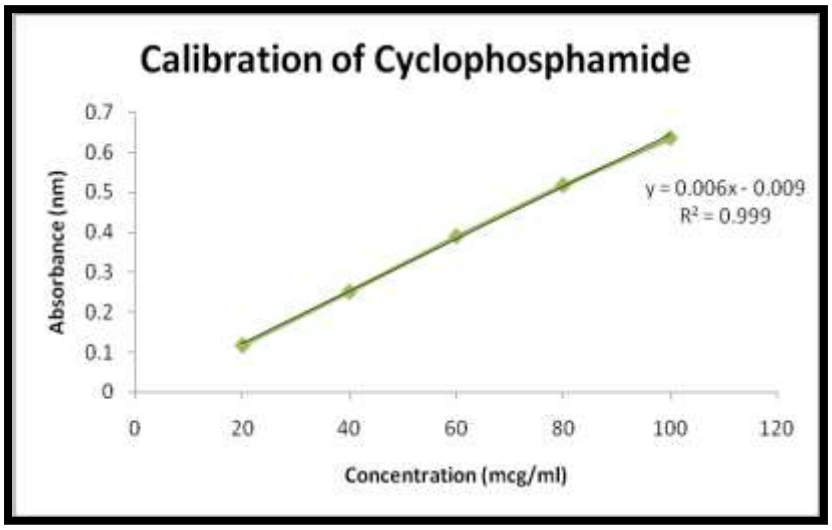


**Figure 2:Calibration curve of Cyclophosphamide in Phosphate buffer pH 7.4**

**Table: Drug release profile of Cyclophosphamide nanoparticles**

| Time (min) | Percentage release (%) |
|---|---|
| 0 | 0 |
| 5 | 29.33±0.342 |
| 10 | 45.67±0.456 |
| 15 | 57.81±0.920 |
| 20 | 69.23±0.471 |

| | |
|---|---|
| 25 | 79.48±0.566 |
| 30 | 86.55±0.890 |
| 60 | 94.52±0.832 |
| 90 | 97.62±0.231 |
| 120 | 98.53±0.522 |

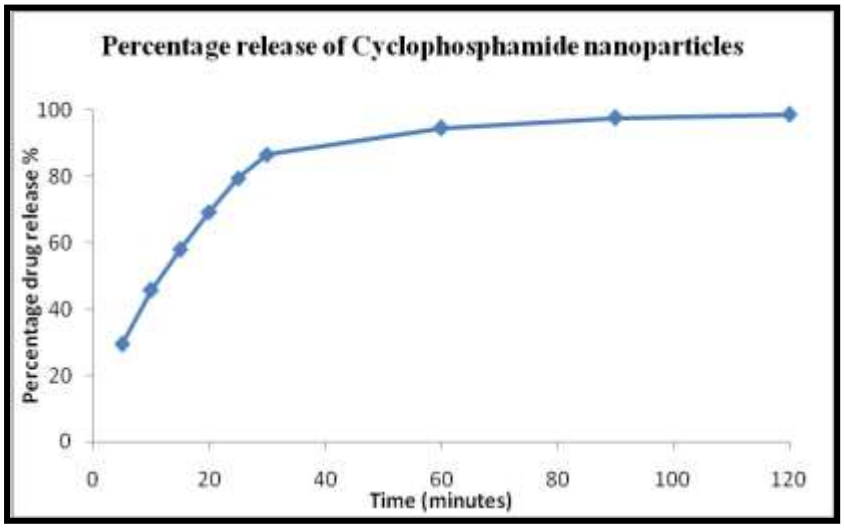


**Figure 3:Percentage drug release of cyclophosphamide**

It has been observed that from the Figure 1&2 the linearity was observed for the drug cyclophosphamide alone in both water and pH 7.4 buffer. The *invitro* dissolution study shows that in Figure 3. about 98.53% of drug release in 2 hours. Nearly half of the drug released at the 10th minute and about 90% release in 30 minutes.

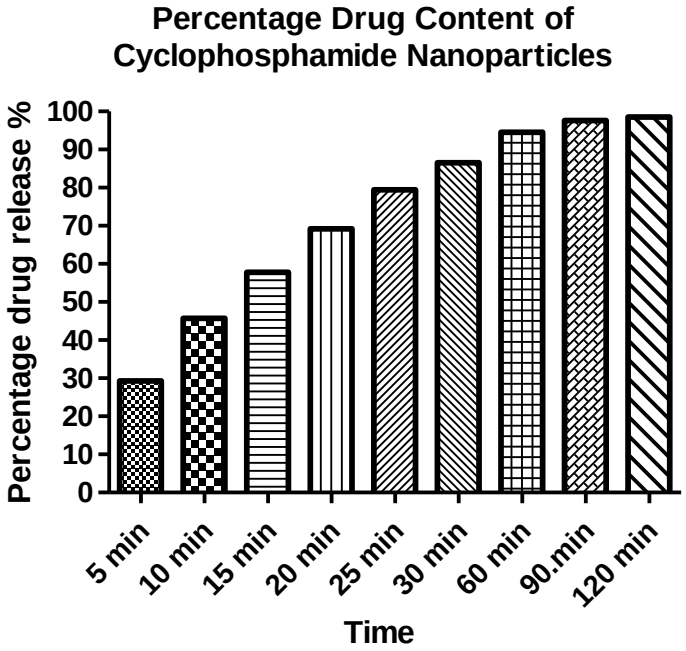


**Figure 4:Scheme of drug release of cyclophosphamide nanoparticle**

**Table 1: Effect of CPG nano extract on relative organ weights of normal control, tumor induced (DAL) and drug treated mice**

| Treatment | Relative Organ Weight (g/100g body wt.) | | | | | |
|---|---|---|---|---|---|---|
| | Body weight (g) | Spleen | Thymus | Liver | Kidney | Lungs |
| Normal control(CMS susp) | 20.48±0.51 | 0.51±0.013 | 0.20±0.012 | 2.86±0.16 | 1.45±0.04 | 0.67±0.03 |
| Tumor induced control ( CMS susp) | 38.46±0.016** | 0.74±0.018** | 0.29±0.016* | 3.76±0.62* | 1.84±0.05ns | 0.78±0.06ns |
| CPG nanoparticles (100 mg/kg)+ DAL | 28.16±0.16*a | 0.68±0.016ns | 0.26±0.011a | 3.41±0.13ns | 1.71±0.07ns | 0.71±0.06ns |
| CP drug (100 mg/kg)+ DAL | 22.43±0.62aaa | 0.49±0.013aa | 0.20±0.017a | 2.98±0.016a | 1.56±0.05a | 0.63±0.06a |

Each Value is SEM of 6 animals * P < 0.05 ; ** P < 0.01 Significance between normal control vs tumor induced control , drug treated group and ;aP< 0.05 ;aa P < 0.01 tumor induced control vs drug treated group NS :Not significant

Group I: Normal Control: Mouse given CMS, intraperitonially (IP) for 15days

Group II: Tumor induced Control: Mouse given CMS ,intraperitonially (IP) for 15 days

Group III: Tumor induced mice treated with CPG Nano extract at the dose of 100 mg/kg body wt, daily (IP) for 15 days

Group IV: Tumor induced mice treated with CP drug at the dose of100 mg/kg body wt, daily (IP) for 15 days

As mentioned in the table 1. a significant reduction in the body weight of the animals where observed with cyclophosphamide drug treated animals as compared with that of the nanoparticles. This shows that the animals under the treatment shown an remarkable reduction in weights.

**Table 2: Antitumor activity of CPG nano extract on solid tumor volume in tumor (DAL)induced mice**

| Treatment Groups | Solid Tumor Volume | | | |
|---|---|---|---|---|
| | $15^{th}$ day | $20^{th}$ day | $25^{th}$ day | $30^{th}$ day |
| Normal control(CMS susp) | - | - | - | - |
| Tumor induced control ( CMS susp) | 6.18±0.12 | 7.16±0.18 | 7.86±0.15 | 8.14±0.16 |
| CPG nanoparticles (100 mg/kg)+ DAL | 4.97±0.24ns | 4.37±0.11** | 3.62±0.54** | 3.08±0.11** |
| CP drug (100 mg/kg)+ DAL | 3.63±0.13** | 3.09±0.15*** | 2.16±0.36*** | 1.84±0.16*** |

Each Value is SEM of 6 animals * P < 0.05; ** P < 0.01 and *** P < 0.001 Significance between tumor induced control vsdrug treated group NS: Not significant

Group I: Normal Control: -Mouse given CMS, intraperitonially (IP) for 15days

Group II: Tumor induced Control: -Mouse given CMS, intraperitonially (IP) for 15 days

Group III: Tumor induced mice treated with CPG Nano extract at the dose of 100 mg/kg body wt, daily (IP) for 15 days

Group IV: Tumor induced mice treated with CP drug at the dose of100 mg/kg body wt, daily (IP) for 15 day

A significant reduction in the tumor cell volume was observed and it was found decreasing gradually on the test dates of $15^{th}$, $20^{th}$, $25^{th}$ and $30^{th}$ days. The drug treated and formulation containing drug as nanoparticles were shown a considerable lessening of tumor cell volume and it is greater with the pure drug as mentioned in the table 2.

**Table 3: Antitumor activity of CPG nano extraction the survival time, life span, tumor volume and viable and non-viable cell count in tumor Induced mice**

| Treatment | Mean Survival time (Days) | Increase of life span(%) | Packed cell volume | Viable cell count X $10^6$ cells/ml | Non-viable tumor cells count X $10^6$cells/ ml |
|---|---|---|---|---|---|
| Normal control (CMS susp) | - | - | - | - | - |
| Tumor induced control (CMS susp) | 19.10±0.16 | - | 4.84±0.13 | 14.54±0.16 | 1.84±0.013 |
| CPG nanoparticles (100 mg/kg)+ DAL | 25.16±0.16* | 35.72 | 3.86±0.16* | 8.56±0.16** | 2.81±0.011ns |
| CP drug (100 mg/kg)+ DAL | 32.16±0.18*** | 68.37 | 2.18±0.16** | 3.36±0.16*** | 3.54±0.015** |

Each Value is SEM of 6 animals * $P < 0.05$ ; ** $P < 0.01$ and *** $P < 0.001$ Significance between tumor induced control vs drug treated group NS :Not significant

Group I :Normal Control : -Mouse given CMS ,intraperitonially(IP) for 15days

Group II :Tumor induced Control : -Mouse given CMS ,intraperitonially(IP) for 15 days

Group III: Tumor induced mice treated with CPG Nano extract at the dose of 100 mg/kg body wt ,daily (IP) for 15 days

Group IV : Tumor induced mice treated with CP drug at the dose of100 mg/kg body wt ,daily (IP) for 15 days

**Table 4: Anticancer activity of CPG nano extract on hematological parameters in tumor(DAL)bearing mice**

| Parameter | Hb (gm%) | RBC (million/mm$^3$) | WBC ($10^3$ cells/ mm$^3$) | Differential count | | |
|---|---|---|---|---|---|---|
| | | | | Lymphocytes | Neutrophils | Eosinophil |
| Normal control(CMS susp) | 13.12±0.19 | 4.54±0.26 | 8.65±0.15 | 55,16±0.11 | 37.26±0.16 | 5.04±0.22 |
| Tumor induced control ( CMS susp) | 9.84±0.56* | 3.06±0.54* | 12.86±0.13* | 42.16±0.36 | 54.16±0.36* | 5.16±0.27 |
| CPG nanoparticles (100 mg/kg)+ DAL | 11.16±0.55ns | 3.89±0.13ns | 10.31±0.54a | 50.16±0.36 | 46.11±0.27 | 3.06±0.31 |
| CP drug (100 mg/kg)+ DAL | 13.63±0.86a | 4.66±0.15aa | 9.13±0.27aa | 52.81±0.76a | 42.16±0.34a | 5.16±0.37ns |

Each Value is SEM of 6 animals * P < 0.05 ; ** P < 0.01 Significance between normal control vs tumor induced control , drug treated group and;aP< 0.05 ;aa P < 0.01 tumor induced control vs drug treated group NS :Not significant

Group I :Normal Control : -Mouse given CMS ,intraperitonially(IP) for 15days

Group II :Tumor induced Control : -Mouse given CMS ,intraperitonially(IP) for 15 days

Group III: Tumor induced mice treated with CPG Nano extract at the dose of 100 mg/kg body wt ,daily (IP) for 15 days

Group IV : Tumor induced mice treated with CP drug at the dose of100 mg/kg body wt ,daily (IP) for 15 days

It has been observed that certain notable changes in the Hb, RBC and WBC counts for the drug treated animals benefited a lot in maintain the normal hematological values as compared with the normal and tumor induced groups

## CONCLUSION

In this present study it is concluded that it is feasible to prepare the nanoparticles containing Cyclophosphamide using Gumghatti as polymer by Nanoprecipitation method. The *invitro* release pattern of the prepared nanoparticle was found to be 98.53% in 2 hours. The antitumor activity of the prepared novel nanoformulation containing Cyclophosphamide as the drug shows a significant reduction in tumor cell volume, body weight and an improvement in hematological parameters. the developed formulation, hence suitable for the better cancer treatment.

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