**METHODOLOGY**

**Formulation of Cyclophosphamide Gumghatti Nanoparticles**
Cyclophosphamide- Gum ghatti Nanoparticles were prepared by dissolving in Gum ghatti Dimethyl sulfoxide (DMSO) and make the drug concentrations of 100mg, 200mg, 300mg, 400mg and 500mg. And it forms the Dispersion with 10 ml of Ethanol (70% for 5 minutes) then evaporates the solvent at 35°C. Then centrifuge at 10000 rpm for 20 min at 4°C then removes the supernatant

**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:

\[ \% \text{Encapsulation Efficiency} = \left( \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \right) \times 100 \]

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.

**Characterization and Evaluation studies of Cyclophosphamide Gumghatti Nanoparticles**
FTIR spectrum of Cyclophosphamide Gum ghatti, Nanoparticles is recorded over the wave region of 500 to 4000 cm\(^{-1}\). The stability of the Nanoparticles system is evaluated by zeta potential measurements and average particle size analysis is performed by DLS-Zeta size Nano series (Malvern). The measurement is done at 25°C for 2 min. The morphology of the Cyclophosphamide -gum ghatti Nanoparticles is examined by scanning electron microscope.

**IMMODULATORY ACTIVITY**
Animals Male Wistar rats weighing 150-180 g were procured from Laboratory Animals Resource from Karpagam College of Pharmacy/ Uwinlab.

a) **Preparation of sheep red blood cells (SRBC)**
From healthy Sheep blood was collected from local butcher house and mixed with sterile Alsever’s solution (1:1). It was thoroughly mixed and centrifuged at 3000 rpm for 5 min. Supernatant was discarded, SRBC pellets were washed with sterilized phosphate buffer saline (pH 7.2) 2-3 times. Then the SRBC pellets were prepared in phosphate
buffer saline (pH 7.2) and total SRBC was counted using Neubauer chamber, finally 1x10^8 SRBCs (0.5ml) confirmed.

b) Animal grouping For experimental procedure, Male Wistar rats were divided in the following four groups containing six rats in each group.

**Group I (n=6):** Negative control: Rats treated with 2 ml of 1% Carboxy Methy Cellulose (CMC) Suspension

**Group II (n=6):** Positive control: Sensitized rats (by administrating 1x10^8 SRBCs (Sheep red blood cells), i. p.) treated with 1% CMC suspension orally.

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

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

SRBCs prepared were injected intra peritoneally for sensitization and challenging the rats. 7days later observe the below parameter:

c) Bone marrow Cellularity

Femur was collected into the medium containing 2% fetal calf serum (FCS). The bone marrow cell number was determined using a hemocytometer and expressed as total live cells/femur.