WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 7, 824-849. Research Article ISSN 2277– 7105

# HERBAL LIPOSOMES WITH EFFECTIVENESS AGAINST DIABETES

**Rajat Singh[1], Shivam[1], Avadh Biharee[2], Deepak Tomar[1], Pankaj Kumar[1], Dheeraj Dubey[1]***

[1]Bhagwati College of Pharmacy, Baraut, Bagpat, Uttar Pradesh, (250611), India.

[2]Pt. Rajendra Prasad Smarak College of Pharmacy, Puranpur, Pilibhit, Uttar Pradesh, (262122), India.

Article Received on
26 April 2021,

Revised on 17 May 2021,
Accepted on 07 June 2021

DOI: https://doi.org/10.17605/OSF.IO/HBDJQ

***Corresponding Author**
**Dheeraj Dubey**
Bhagwati College of Pharmacy, Baraut, Bagpat, Uttar Pradesh, (250611), India.

## ABSTRACT

The main motive behind this study was to formulate an herbal drug loaded nanosized carrier which enhances the permeability of a hydrophilic herbal drug through the membrane and increases the drug concentration into the systemic circulation. And second motive is to provide a cheap formulation to the future consumers which will be deal with the diabetes on that time. An alternative of that drug was silver nanoparticles present but they are very costly at that time. The effectiveness of the formulation was considered on the basis of the In-Vitro inhibition study of the alpha-amylase by *Pterocarpus marsupium* loaded liposomes and compared with a positive control acarbose and it was shows that high concentration of drug was as effective as acarbose to inhibit the alpha – amylase. In the end of the study animal testing was done which shows effectiveness of the formulation as compared to the plane and uncovered *Pterocarpus marsupium.*

**KEYWORDS:** Liposomes, diabetes, *Pterocarpus marsupium*, Alloxan.

## INTRODUCTION

Diabetes have the status of a budding epidemic in India, more than 62 million people were currently diagnosed with this disease. Basically, it is a bundle of chronic disorder of fat, Carbohydrate and protein metabolism which can cause increased fasting and post prandial blood sugar levels. Globally diabetes is approximated to raise from 4% in 1995 to 5.4% by the year 2025.

Diabetes mellitus is also known as diabetes because it is a group of various metabolic disorders and their symptoms are high blood sugar include with frequent urination, increased hunger and increased thirst. When they leave untreated, they will cause many obstacles like diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death and some sever long-term stumbles which are cardiovascular disease, stroke, chronic kidney disease, damage to the eyes, and foot ulcer. (Salih, Karem, & Jassim, 2019)

There are basically three types of diabetes mellitus which are as follows:

1. Type 1 Diabetes Mellitus
2. Type 2 Diabetes Mellitus
3. Gestational diabetes

**Use of Herbal medication for treatment purpose:** Due to the increased focus of people towards herbal substance and the traditional evidences of the herbal plants and herbs for the treatment of various diseases. For the treatment of diabetes there are various plants are available in which some of the following as follows.

- Holy Fruit tree (Aegle marmelos)
- Onion (Allium cepa) & Garlic (Allium Stivum)
- Japanese angelica tree (Aralia elata)
- Neem (Azadirachta indica)
- Orchid tree (Bauhinia candicans & B. forficte)
- Little tree plant (Biophytum sensitivum)
- Black Mustard (Brassica nigra)
- Cinnamon (Cinnamomum zeylanicum)
- Huanglian (Coptis chinensis)
- Guar (Cyamopsis tetragonoloba)
- Java plum (Eugenia jambolana)
- Banyan (Ficus bengalenesis)
- Potato (Grewia asiatica)
- Gurmar (Gymnema sylvestre)
- Henna (Lawsonia inermis)
- Purple lossestrife (Lythrum salicaria)
- Alfalfa or Lucerne (Medicago sutivu)

- Bitter Melon (Momordica charantia)
- Mulberry (Morus alba)
- Curry leaf (Murraya koeingii)
- Holy basil (Ocimum sanctum)
- Ginseng root (Panax ginseng)
- Kutki (Picrorrhiza Kurroa)
- Rhizoma (Polygonati odorati)
- Guava (Psidium guajava)
- Vijayasar (Pterocarpus marsupium)
- Gaduchi (Tinospora cordifolia)
- Fenugreek Seeds (Trigonella foenumgraecum)
- Ginger (Zingiber officinale)
- Jamun (Syzygium cuminii) etc.

**Morphology of Vijayasar (*Pterocarpus marsupium)***

It's a large deciduous tree that reaches a height of 90 feet or more.

**Bark:** The stem bark is grey-brown to brown in colour. The heartwood is a golden yellow colour, and the bark has a reddish gum.

**Leaves:** 3 - 5 inches long, with 5 - 7 leaflets and a wavy, obtuse margin. Petioles are 5 or 6 inches long, round, smooth, and waved from leaflet to leaflet, with no stipules.

flower: 1.5 cm long, multiple, white with a slight yellow tinge The stamens are ten in number, united near the base but soon dividing into two parcels of five each; the other stamens are globose and two lobed.

**legume**: The lower third of the style, which extends from the pedicel to the remainder of the style, is bicular; the upper third, which extends from the pedicel to the remainder of the style, is straight; the whole is enclosed by a waved, veiny, downy, membraneous wing, swollen, rugose, woody in the middle, where the seed is lodged and not opening; (Gairola, Gupta, Singh, Maithani, & Bansal, 2010).

It's made up of small, irregular pieces of bark that vary in size and thickness. Its colour is golden yellowish-brown with darker stripes. It is extremely fragile and heavy. It produces a yellow-colored solution with a blue fluorescence when dissolved in water. Tracheids, fibre tracheids, xylem parenchyma, and xylem rays are represented in a transverse section as alternating bands of larger and smaller polygonal cells. Xylem vessels can be found all over

the body. There are tannin-filled tyloses present. Tracheids are long, thick-walled, and have simple pits at the ends. The xylem parenchyma cells are rectangular with simple pits and uni-to-biseriate xylem rays. There are calcium oxalate crystals present, but no starch, starch granules, or oil globules. (Gairola et al., 2010)

**Scientific Classification**

Synonyms: Indian kino, Bijasal, Vijayasar, Bibla.

Family: Fabaceae

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridaeplantae

Phylum: Magnoliophyta

Subphylum: Euphyllophytina

Infraphylum: Radiatopsis

Class: Magnoliopsida

Subclass: Rosidae

Super order: Fabanae

Order: Fabales

Genus: Pterocarpus

Species: marsupium (Devgun, Nanda, & Ansari, 2009)

**Chemical Constituents**

Pterocarpus is known to be a rich source of polyphenolic compounds, according to previous research. Pterocarpus marsupium's active concepts are all thermostable It's made up of pterostilbene 4-5%, alkaloids 0.4%, tannins 5%, protein, pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, P-hydroxybenzaldehyde, Beudesmol, erythrodirol-3-monoacetate, l-epicatechin, marsupinol, irisolidone-7-O-A-L-rhamnopyranoside, have primarily come from the heartwood and root.

Gum kino from the bark provides non-glucosidal tannins.

- Kinotannic acid
- Kinonin (C28H24O12)
- Kino-red (C28H22O11)
- Pyrocatechin
- Pyrocatechin acid & small quantities of resin, pectin and gallic acid.(Maurya et al., 2004)

Aqueous extract of the heartwood of Pterocarpus marsupium contains 5 new flavonoids C-glucosides namely 6-hydroxyl-2-(4-hydroxybenzyl)-benzo-furan-7-C-â-D-glucpyranoside, 3-(á-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3H)-furanone-7-C-â-D-glucopyranoside, 2-glucopyranoside, 8-(C-â-D-glucopyranosyl)-7,3,4-trihydroxyflavone and 1,2-bis (2,4-dihydroxy, 3-C-glucopyranosyl)-ethanedione and two known compounds C-â-D-glucopyranosyl-2,6-dihydroxyl benzene and sequiterpene were isolate(Handa et al., 2000)

## AIM AND OBJECTIVES OF THIS STUDY

The purpose of this research is "Herbal Liposome's with effectiveness against Diabetes" Diabetes is a bundle of chronic disorder of fat, Carbohydrate and protein metabolism which can cause increased fasting and post prandial blood sugar levels. The goal of treatment of diabetes may be true of that can be just, often general causes of diabetes are examination tension, overweight, not proper intake of food and also high blood pressure , the incident that had already occurred, lot of tension about family and may be job among them. A normal part of life is worry, doubt and over tension. *Pterocarpus marsupium* plant has shown effective results in the treatment of diabetes, anti-glycemic, antioxidant and anti-inflammatory based on literature survey. The main objectives of current study are -

- Collection and authentication of *Pterocarpus marsupium*.
- Extraction of hydro ethanolic extract of *Pterocarpus marsupium*.
- Formulation development of *Pterocarpus marsupium*.
- To evaluate the effectiveness against diabetes via liposome's of *Pterocarpus marsupium*.

## MATERIAL AND METHODOLOGY

**Plant collection and authentication:** The whole plant or their parts shall be collected and authenticated by a Ch. Chhoturam (PG) College, Muzaffarnagar, U.P. of Ref. No. BOT/AUTH./10 authority in plant taxonomy. The identified and authenticated species were be collected in sufficient quantity, dried and powdered for further studies.

**Preparation of ethanolic extract of *Pterocarpus marsupium:*** The shade dried bark of *Pterocarpus marsupium* was powdered with the help of grinder. Extraction was performed by packing the coarsely powdered (85mg) drug in soxhlet assembly (with round bottom flask containing hydro ethanolic as solvent and reflux condenser on top). Round bottom flask were kept on heating mantle at the temperature up to $55^0$C. It does not exceed as MERK INDEX says that the BP of ethanol at 760mm of hg is 64 $^0$C. After that solvent was removed through evaporation and then a semi-solid extract was obtained to further liposome's formulation.

**Animals:** All experiments were performed in the year of 2019, using healthy rats of either sex (200-600 gm) collected from the Department of Pharmacy's animal house, Meerut Institute of Engineering and Technology, Meerut, Uttar Pradesh 250005 and animals held at ambient temperature of 25±2 $^{0}$C and 55-65 relative humidity with 12 hours light and dark period. The animals had free access to stand tap water and pellet chow. Animals were kept in groups for at least a week before they were used for experimentation.

**Drugs and Chemicals:** Metformin as normal medication, distilled water, hydro ethanol and liposomes formulation as a test drugs.

**Glassware and Apparatus:** Measuring cylinder, funnel, beaker, glass rod, soxhlet apparatus, round bottom flask, condenser, heating mantle and balance weighing.

**Experimental Protocol**

**Treatment:** The present study employed five groups and each group consisting of six rats of both sexes as mention below. All the solutions were freshly prepared and administered in animals by intraperitoneal and oral route.

**List of handled groups:** To evaluate the diabetes activity of *Pterocarpus marsupium* in rats all the selected animals are divided in four groups and the list of groups are given in Table 7.1.

**Table 1: List of Animal group for anxiolytic activity evaluation.**

| Groups | Treatment |
|---|---|
| Group A | 1 ml/100 gm body weight, orally. |
| Group B | 5 % aqueous solution of metformin orally through a gastric tube (50 mg/100gm, orally). |
| Group C | 5% aqueous solution of *Pterocarpus marsupium* liposomes at a dosage of 50 mg/100 gm body weight, orally. |
| Group D | 5% aqueous solution of *Pterocarpus marsupium* liposomes at a dosage of 50 mg/100 gm body weight, orally. |

**METHOD OF FORMULATION DEVELOPMENT**

**Preparation of Blank Liposomes:** The preparation of liposomes based on the bath sonication method. For the preparation of liposomes Cholesterol, Chloroform, Phospholipids, & Phosphate buffer saline having a pH 7.4 was taken and weighed accurately as described in table and then mix cholesterol, Phospholipids and cholesterol in a clean beaker uniformly and then add this mixture into the Phosphate buffer saline having pH 7.4 with continues steering

and then transfer the mixture into rotary flash evaporator at 60$^{o}$C to evaporate the whole chloroform to make SUV type of liposomes but the vesicle size may be big so that for the reduction of size bath sonication for 30 min was done.


**Fig. 1: Blank liposomes.**

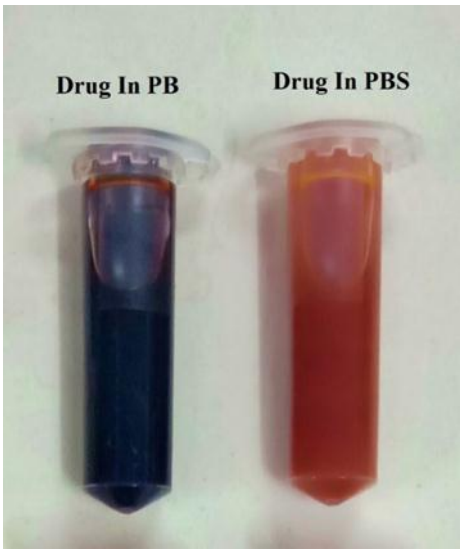


**Fig. 2: Drug incorporation in PB and PBS solutions.**

**Preparation of Drug Loaded Liposomes:** It was also based on the bath sonication method. For the preparation of the drug loaded liposomes extract of Pterocarpus marsupium, Cholesterol, Chloroform, Phospholipids, & Phosphate buffer saline and Phosphate buffer both having pH 7.4 was taken and weighed accurately as described in the table and then two batches of the liposomes was prepared in which one was prepared in Phosphate buffer saline and second was prepared in Phosphate Buffer having pH 7.4 in this extract of Pterocarpus

Marsupium, cholesterol, chloroform, Phospholipids mixed in two different beakers and then add into both buffer solutions respectively with continuous stirring and then transfer both batches one by one into the rotary flash evaporator at 60$^{o}$C to evaporate the whole chloroform to make SUV type of liposomes but the vesicles size may be big so that for the reduction of size was done by Bath Sonicator for 30 min.

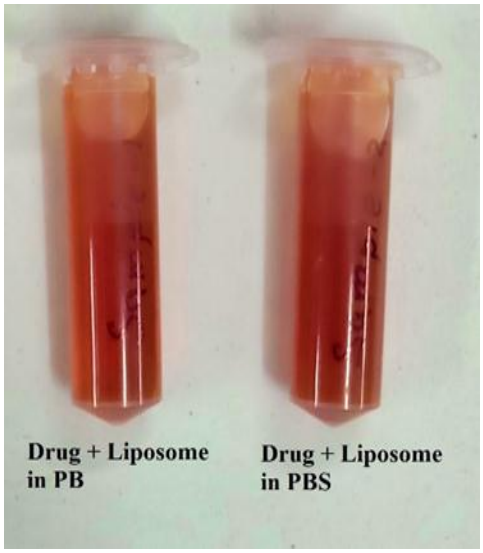


**Fig. 3: Developed batches of liposomes of Pterocarpus marsupium in PB and PBS solutions respectively.**

**Table 2: Batch preparation list for formulation development.**

| S.No. | Formulation | Lecithin | Cholesterol | Chloroform | DOPC | DPPC | Drug | PB solution | PBS Solution |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Blank | 134mg | 67mg | 7ml | 8.3mg | 8.3mg | - | 100ml | - |
| 2 | Drug in PB | - | - | - | - | - | 2gm | 100ml | - |
| 3 | Drug in PBS | - | - | - | - | - | 2gm | - | 100ml |
| 4 | F1 | 134mg | 67mg | 7ml | 8.3mg | 8.3mg | 4gm | 150ml | - |
| 5 | F2 | 134mg | 67mg | 7ml | 8.3mg | 8.3mg | 4gm | - | 150ml |

**Inference:** According to this table various formulations was developed for the treatment of diabetes which was evaluated by using various evaluation parameters.

**Characterization of Pterocarpus marsupium loaded liposomes**

**FTIR spectroscopy of blank liposomes:** 50 mg of dried form of liposomes mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000-400 cm-1 range.

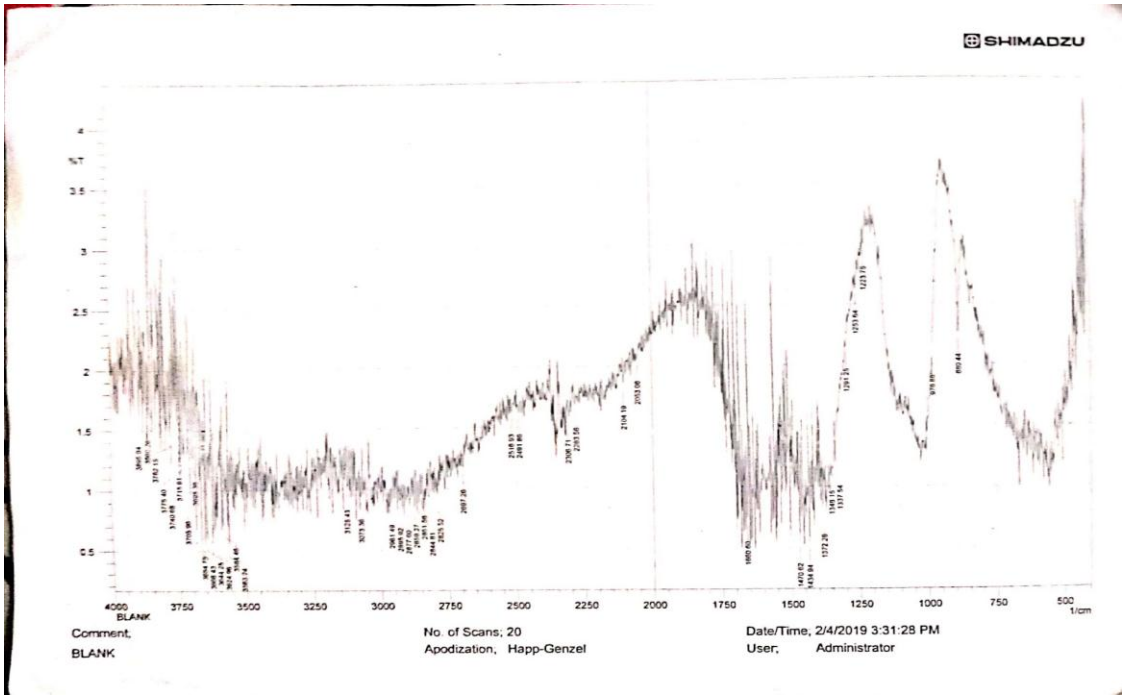


**Fig. 4: FTIR spectra of blank liposomes.**

**Interpretation of Blank liposomes**

| Functional Groups | Wavenumber ($cm^{-1}$) |
|---|---|
| ≡C-H- | 2851.58 |
| C=C-H- | 3073.36 |
| C-H Aromatic | 3073.36 |
| O=C-H- Aldehyde- | 2697.26 |
| -OH- Phenols- | 3624.96-3644.25 |
| -OH -Hydrogen bonded alcohol- | 3230-3390.98 |
| ≡ N-H- Amines | 3350-3501.38 |
| -C-O- Alcohols, ethers- | 1223.75-1291.25 |
| ≡C-N-Amines- | 1223.75-1348.15 |
| >C=C<Alkenes- | 1660.60 |
| -NO2-Nitro compound- | 1337.54-1470.62 |
| C-H- Stretching- | 2851.56-2961.49 |

**Inference:** According to this spectral analysis there are long chain polymers was present with some aldehyde, amines, alcoholic ethers, OH bonds, Nitro compounds etc. In this spectra various double and triple bonded carbon molecules in chain form was present.

**FTIR spectroscopy of Pterocarpus marsupium extract loaded liposomes:** Both batches were taken for the FTIR spectral. 50 mg of dried form of both samples of Pterocarpus marsupium extract loaded liposomes mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000-400 cm-1 range.

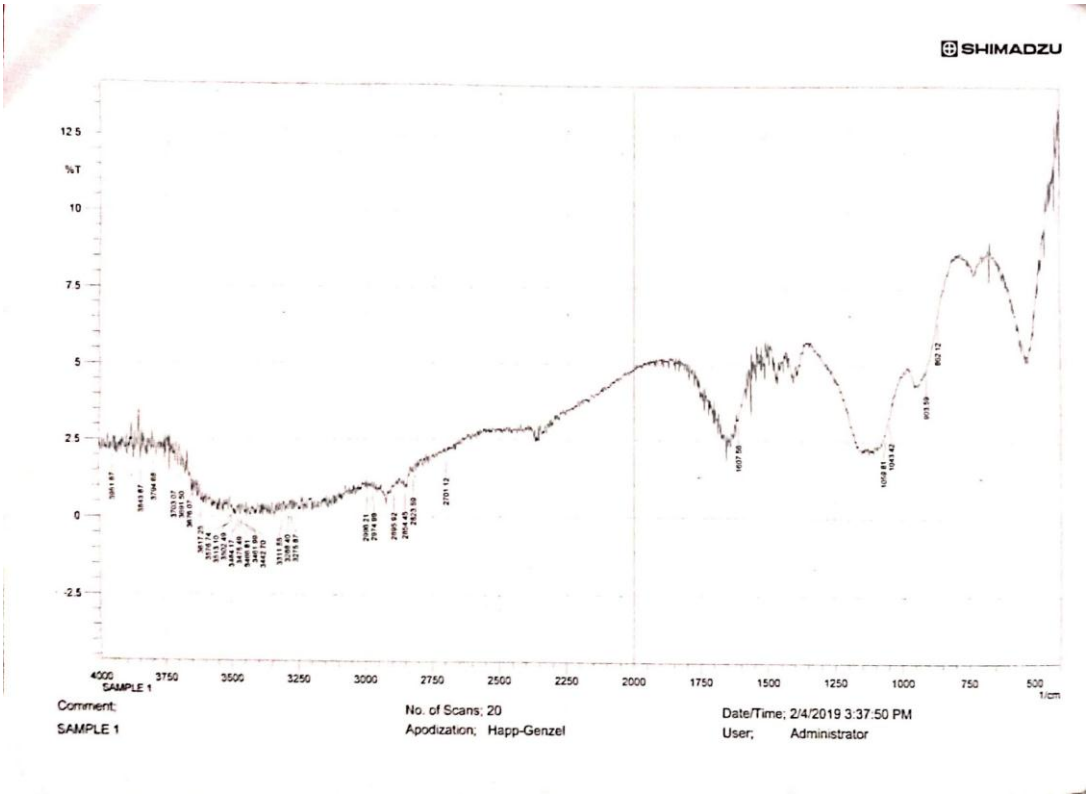


**Fig. 5: FTIR spectra of the drug loaded liposomes in PB solution.**

**Interpretation of Drug loaded liposomes in PB.**

| Functional Groups | Wavenumber (cm-1) |
|---|---|
| ≡C-H- | 2854.59- 2895.92 |
| C=C-H- | 3030-3095.98 |
| C-H Aromatic | 3030-3095.98 |
| O=C-H- Aldehyde- | 2666.53-2854.45 |
| -OH- Phenols- | 3617.25 |
| -OH -Hydrogen bonded alcohol- | 3275.87-3311.55 |
| ≡ N-H- Amines | 3311.55-3502.49 |
| -C-O- Alcohols, ethers- | 1043.42-1059.81 |
| C-H- Stretching- | 2895.92 |

**Inference:** According to this spectral analysis some basic components like aromatic ring aldehyde, phenolic compounds, OH bonds, amines etc was matched with the normal drug spectra and some traces of the long chain molecules were present in it. It was show that there was no interaction between drug and polymer.

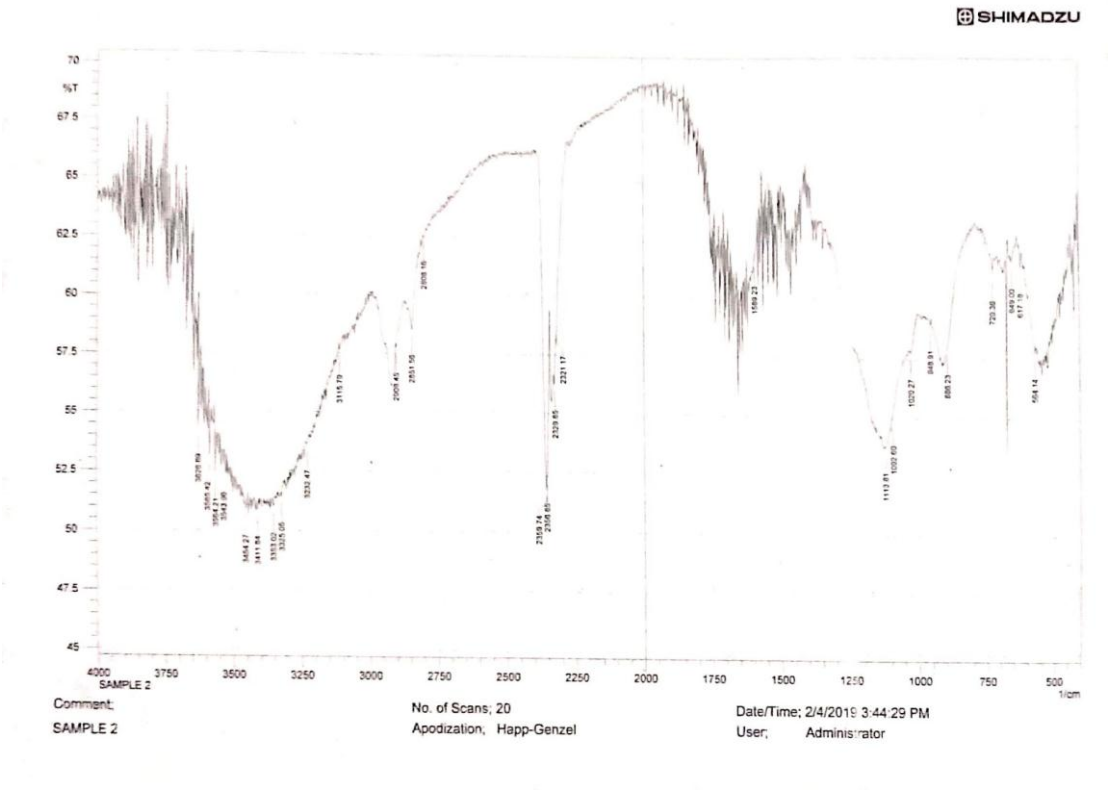


**Fig. 6: FTIR spectra of drug loaded liposomes in PBS solution.**

**Interpretation of Drug loaded liposomes in PBS.**

| Functional Groups | Wavenumber (cm-1) |
|---|---|
| ≡C-H- | 2823.59 |
| =C-H- | 3030.93-3083.96 |
| C≡C-H- | 3300.94 |
| C-H Aromatic – | 2988.49-3083.49 |
| O=C-H- Aldehyde- | 2767.66-2853.49 |
| O-C-O- ketone- | 1090.67 |
| -OH Phenols- | 3605.67 |
| -OH- Hydrogen bonded alcohol- | 3237.29-3419.56 |
| ≡N-H- Amines- | 3300.94-3501.52 |
| -C-C- Aromatic ring- | 1515.39- 1565.83 |
| C-H Stretching- | 2823.59-2918.10 |

**Inference:** According to the spectral analysis of this sample was observed that it contains the aromatic, phenolic compounds with some aldehyde, ketones and amines which was matched with the drug spectra and some other elements like long chain acids and carbon double and triple bonds was present. It was show that there was no interaction between drug and polymer with respect to this spectral analysis.

**Percentage of Drug Entrapped**

Total Amount of Pterocarpus marsupium extract = 8 gm which is divided into two equal parts 4 + 4 gm which were further divided in to 3 small batches of 1.3 gm each to make 6 different batches so that the percentage of drug entrapped in liposomes was.

**Liposomes in Phosphate buffer**

**Table 3: Percentage encapsulation of batch 1.**

| S.No | Sample size(gm) | Wavelength | Conc in gm/150 ml |
|---|---|---|---|
| 1 | 1.3 | 3.010 | 0.691 |
| **2** | 1.3 | 3.020 | 0.693 |
| **3** | 1.3 | 3.005 | 0.690 |
| **Mean** | | | 0.691 |

Amount of unbound drug in the phosphate buffer: 0.69gm/ 150 ml

So that the percentage of drug entrapment = $\frac{4-0.69}{4} * 100$

= 82.7%

**Liposomes in Phosphate buffer saline**

**Table 4: Percentage encapsulation of batch 2.**

| S.No | Sample size(gm) | Wavelength | Conc in gm/150 ml |
|---|---|---|---|
| 1 | 1.3 | 2.552 | 0.585 |
| **2** | 1.3 | 2.563 | 0.588 |
| **3** | 1.3 | 2.545 | 0.583 |
| **Mean** | | | O.585 |

Amount of unbound drug in Phosphate buffer saline: 0.59gm/150ml

So that the percentage of drug entrapment = $\frac{4-0.59}{4} * 100$

= 85.25%

**Inference:** This study shows that about 4 gm of the drug was incorporated into the liposomal formation in which about 0.69 and 0.59gm of drug was still remain outside the in PB and PBS respectively. So that about 3.31gm in PB and 3.41gm in PBS was founded to be bound.

**Permeation study**

**Table 5: Permeation rate of drug through membrane with respect time of formulation 1 in PB.**

| Time (min) | First Batch | Second Batch | Third Batch | Mean |
|---|---|---|---|---|
| 0 | 3.99 | 3.43 | 3.9 | 3.773333333±11.30999 |
| 30 | 12.79 | 12.65 | 12.81 | 12.75±11.30999 |
| 60 | 29.22 | 29 | 29.91 | 29.37667±11.30999 |
| 90 | 33.42 | 32.96 | 33 | 33.12666667±11.30999 |
| 120 | 27.46 | 28 | 27.66 | 27.70666667±11.30999 |
| 150 | 25.38 | 25.67 | 24.91 | 25.32±11.30999 |
| Mean | | | | 22.00889±11.30999 |

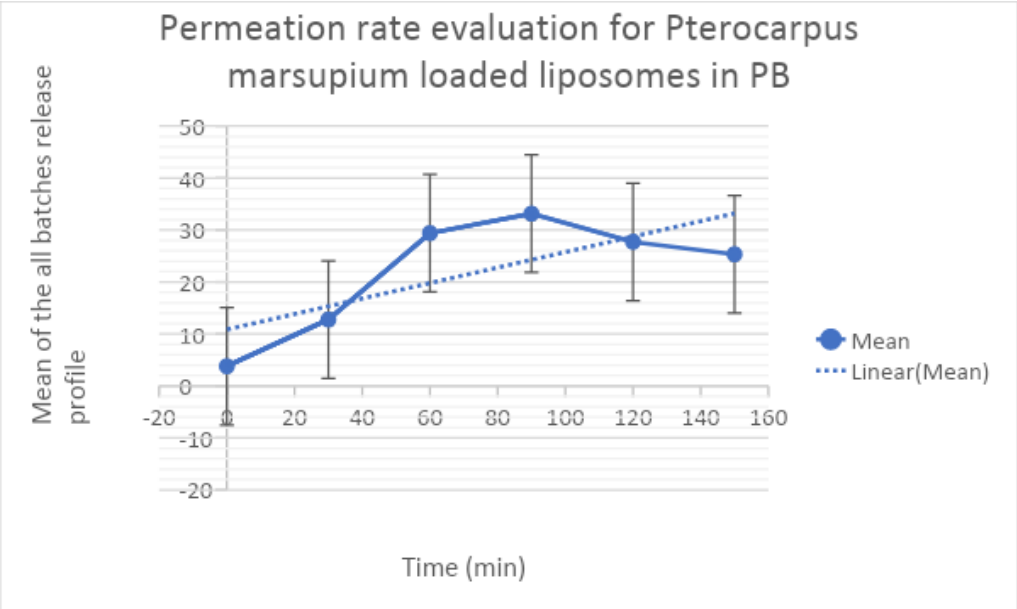


**Fig. 7: Permeation rate of *Pterocarpus marsupium* liposomes in Phosphate buffer.**

**Table 6: Permeation rate of drug through membrane with respect time of formulation 2 in PBS.**

| Time (min) | First Batch | Second Batch | Third Batch | Mean |
|---|---|---|---|---|
| 0 | 2.99 | 2.54 | 2.9 | 2.81±11.41885 |
| 30 | 11.79 | 11.65 | 11.81 | 11.75±11.41885 |
| 60 | 27.22 | 27.66 | 27.91 | 27.59666667±11.41885 |
| 90 | 32.42 | 32.96 | 33.62 | 33±11.41885 |
| 120 | 26.46 | 27.56 | 26.66 | 26.89333333±11.41885 |
| 150 | 24.38 | 24.67 | 24.91 | 24.65333333±11.41885 |
| Mean | | | | 21.11722222±11.41885 |

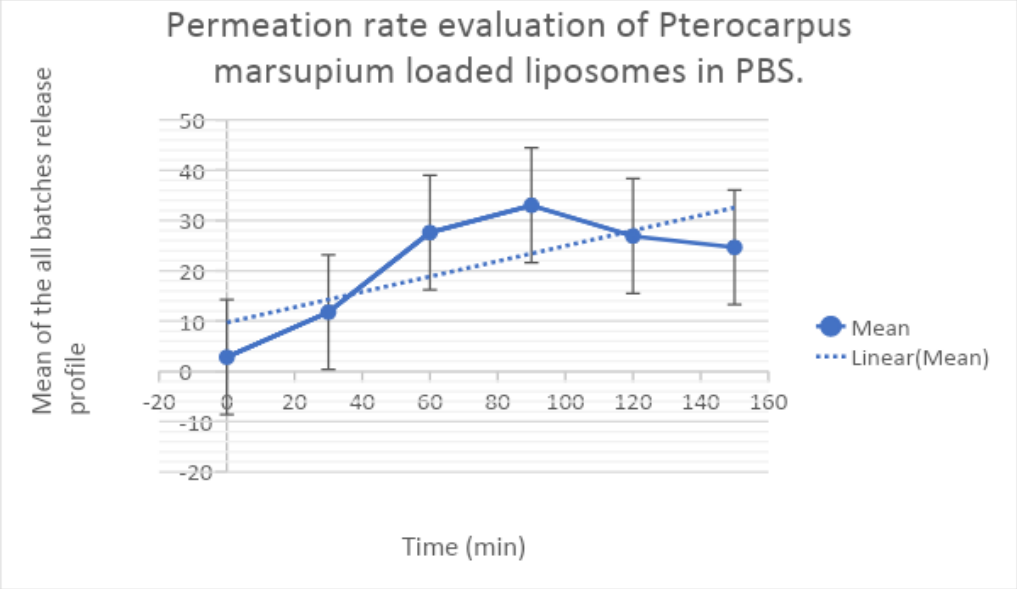


**Fig. 8: Permeation rate of *Pterocarpus marsupium* liposomes in Phosphate Buffer saline.**

**Inference:** After taking the U.V. Spectra of the release profile show that at the initial time period the drug concentration was increased showing first order non-linear kinetics up to reaching a time period 1:30 hr. after that a liner decrease the drug concentration. The reason behind to perform this study to examine the enhancement of the permeation rate of the Pterocarpus marsupium in the liposomal suspension form membrane to achieve the better absorption of drug though membrane by enhancing permeation rate.

**Determination of particle size**: The particle size of Pterocarpus marsupium loaded liposomes can be measured by under optical microscopy (Quasmo Phase contrast microscopy) to evaluate the formation of liposomes and the particle size was determined by Malvern zeta size analyser.

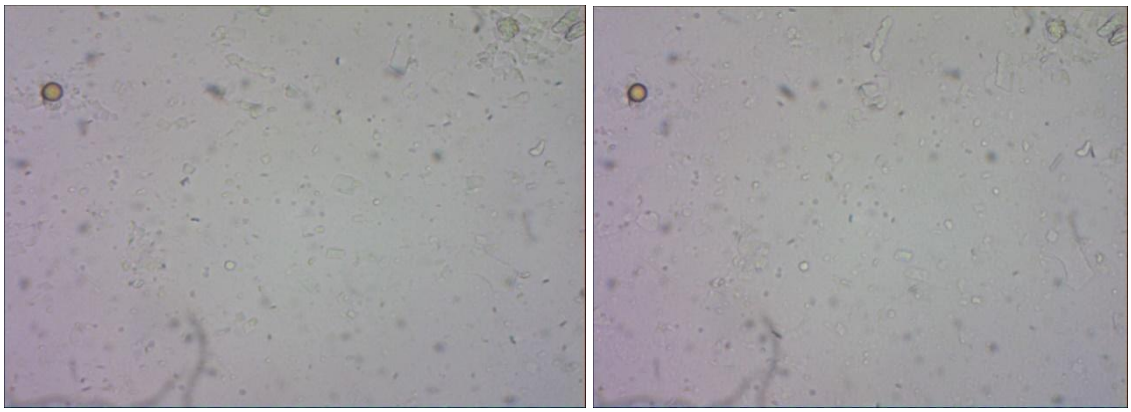

**Fig 9: Microscopic detection at 45X magnification for Formulation 1 in PB.**

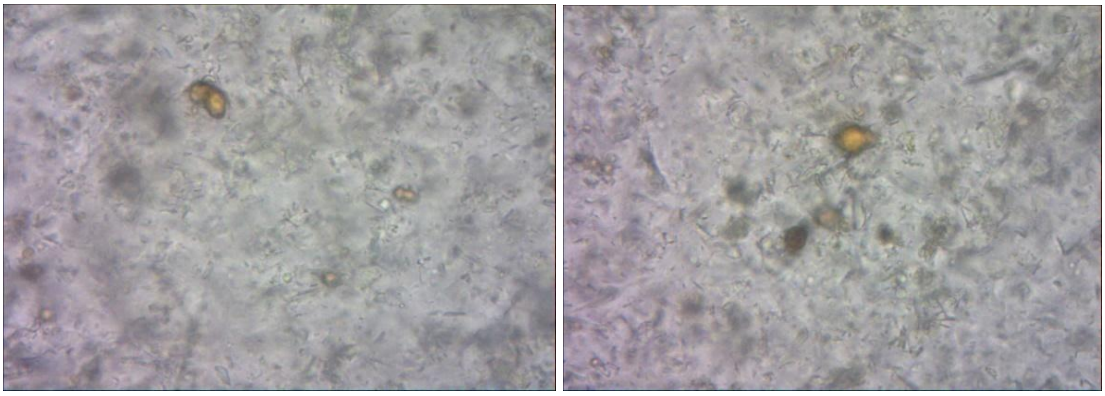

**Fig. 10: Microscopic detection at 45X magnification for formulation 2 in PBS.**

After taking a number of images and calculating the size of the liposomes which are able to see through the optical microscope being calculated by using stage micrometer size calculation method.

**Table 7: Calculation for size detection of liposomes by using Stage micrometer method.**

| Scope particle size | magnification factor | Actual size in um |
|---|---|---|
| 11 | 15 | 165 |
| 15 | 15 | 225 |
| 10 | 15 | 150 |
| 10 | 15 | 150 |
| 10 | 15 | 150 |

But some liposomes were in the nano range which would be identified by using Malvern Zeta sizer which was as follows.

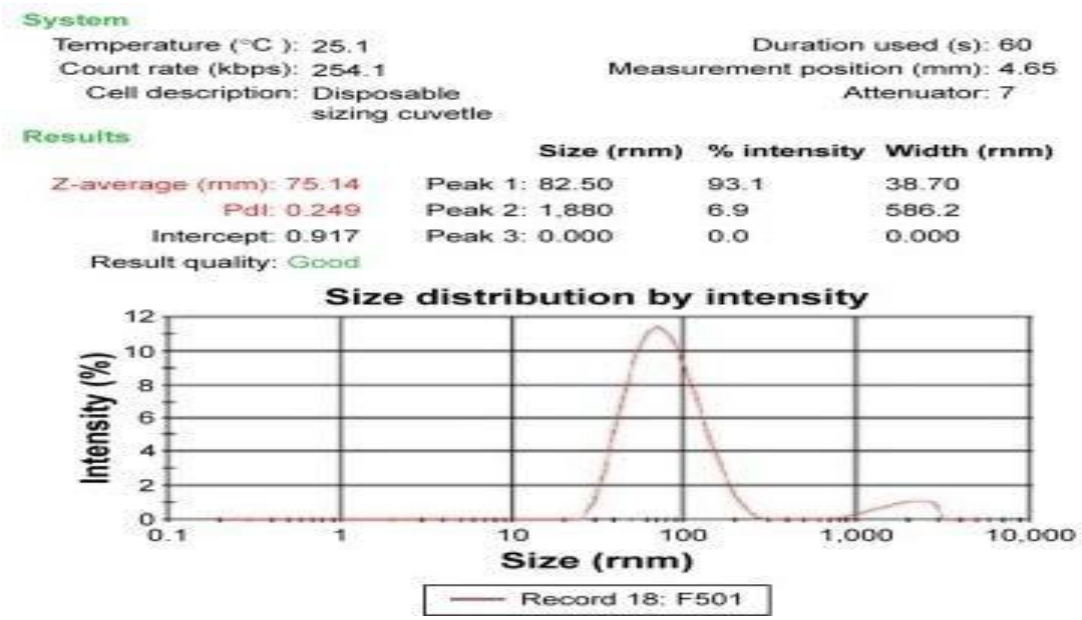


**Fig. 11: Malvern Zeta sizer graph for size detection of *Pterocarpus marsupium* loaded liposomes in PB solution.**

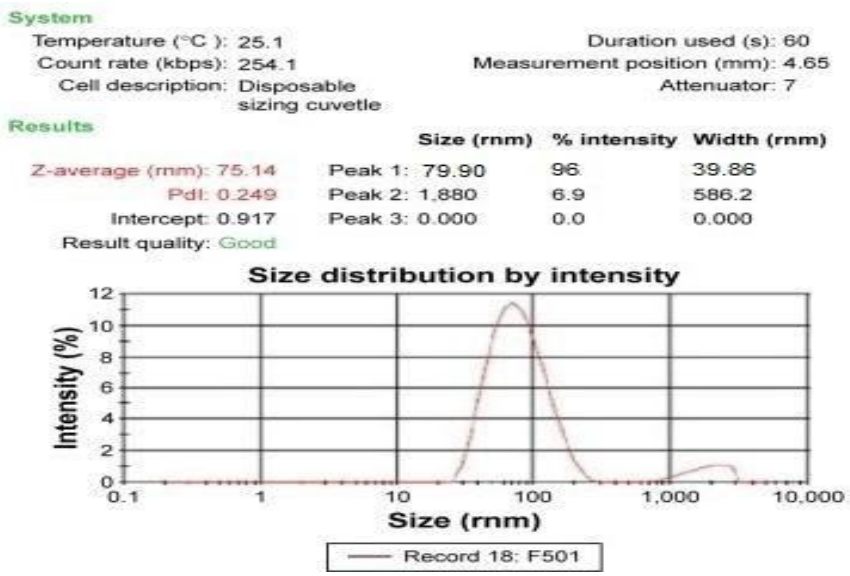


**Fig. 12: Malvern zeta sizer graph for size detection of the *Pterocarpus marsupium* loaded liposomes in PBS solution.**

**Inference**: According to the Optical microscopy (45X magnification) of the both samples was observed 150-225 um range of some liposomal droplets but major liposomes were present in nano range which was calculated by Malvern Zeta sizer the peaks shows the first region of about radius 82.50nm and width about 38.70nm which shows the maximum range 93.1 % of liposomes in PB solution and According to Malvern Zeta sizer the peaks shows the first region of about radius 79.90nm and width about 39.86nm which shows the maximum range 96% of liposomes in PBS.

**Determination of zeta potential**

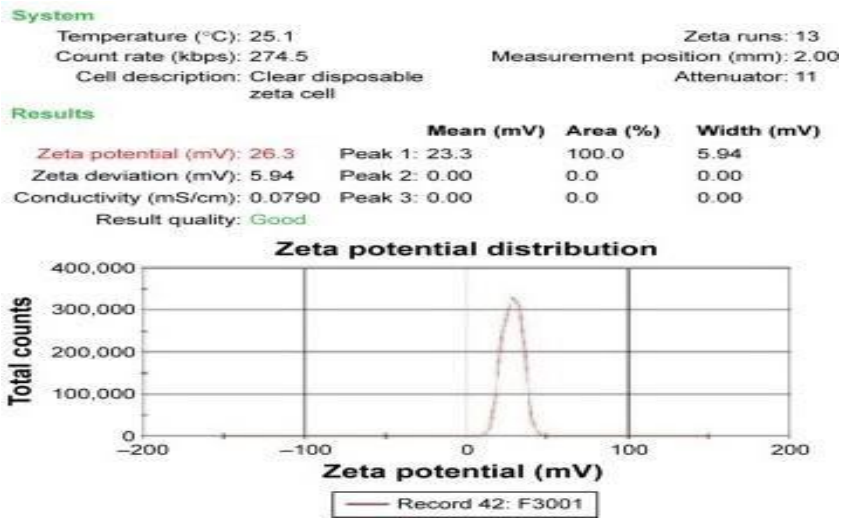


**Fig. 13: Malvern Zeta potential for evaluating the potential of the drug loaded liposomes in PB solution.**

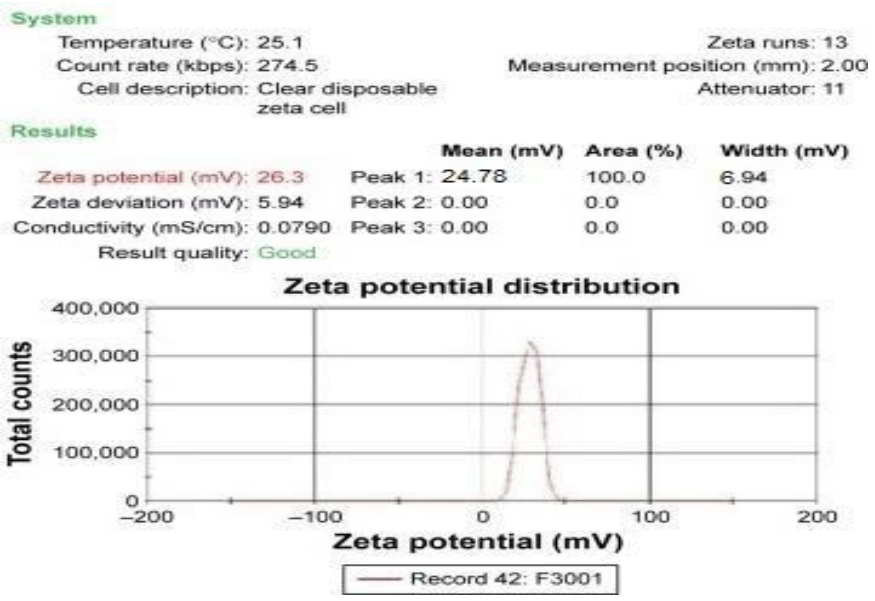


**Fig. 14: Malvern Zeta Potential for evaluating Potential of the drug loaded liposomes in PBS solution.**

**Inference:** According to the Malvern Zeta potential the mean Potential of the sample 1 was 26.3mV and the mean first peak potential was 23.3mV and width was 5.94mV for liposomes in PB solution and according to the Malvern Zeta Potential the mean potential of the sample 2 was 26.3mV and the mean of the first peak was 24.78 and its width was 6.94mV for liposomes in PBS solution.

### Stability Studies

The stability of the Pterocarpus marsupium extract encapsulated liposomes was evaluated after storage at 2-8$^{o}$C, room temperature and 45$^{o}$C for three months. The particle size distribution and drug encapsulation efficiency of the samples were determined as a function of the storage time.

### Batch One

**Table 8: Stability study of the *Pterocarpus marsupium* loaded liposomes in PB solution.**

| Parameters | Changes due to storage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Within 15 days | | | Within 1 month | | | Within 3 months | | |
| | 2-8$^{o}$C | RT | 45$^{o}$C | 2-8$^{o}$C | RT | 45$^{o}$C | 2-8$^{o}$C | RT | 45$^{o}$C |
| Drug Remaining | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.3 | 3.3 | 3.3 |
| pH | 7.2 | 7.1 | 7.3 | 6.8 | 6.7 | 6.9 | 6.3 | 6.7 | 6.8 |
| Color Change | No change | No change | No change | No change | No change | No change | No change | No change | No change |

**Batch Two**

**Table 9: Stability study of Pterocarpus marsupium loaded liposomes in PBS solution.**

| Parameters | Changes due to storage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Within 15 days | | | Within 1 month | | | Within 3 months | | |
| | 2-8°C | RT | 45°C | 2-8°C | RT | 45°C | 2-8°C | RT | 45°C |
| Drug Remaining | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| pH | 7.4 | 7.4 | 7.4 | 7.2 | 7.3 | 7.3 | 7.1 | 7.2 | 7.0 |
| Color Change | No change | No change | No change | No change | No change | No change | No change | No change | No change |

**Inference:** In between the duration of stability study of the sample products there was some slightly changes in concentration were occur which was evaluated by using UV method and there were also change in pH but not change in color.

**In-Vitro study of Pterocarpus marsupium loaded liposomes**

**Table 10: percentage inhibition of α Amylase by Acarbose.**

| Concentration (ug/ml) | Absorbance | Percentage inhibition |
|---|---|---|
| 50 | 0.832 | 41.44% |
| 100 | 0.703 | 50.52% |
| 200 | 0.625 | 56.01% |
| 400 | 0.426 | 70.02% |
| 800 | 0.226 | 84.09% |

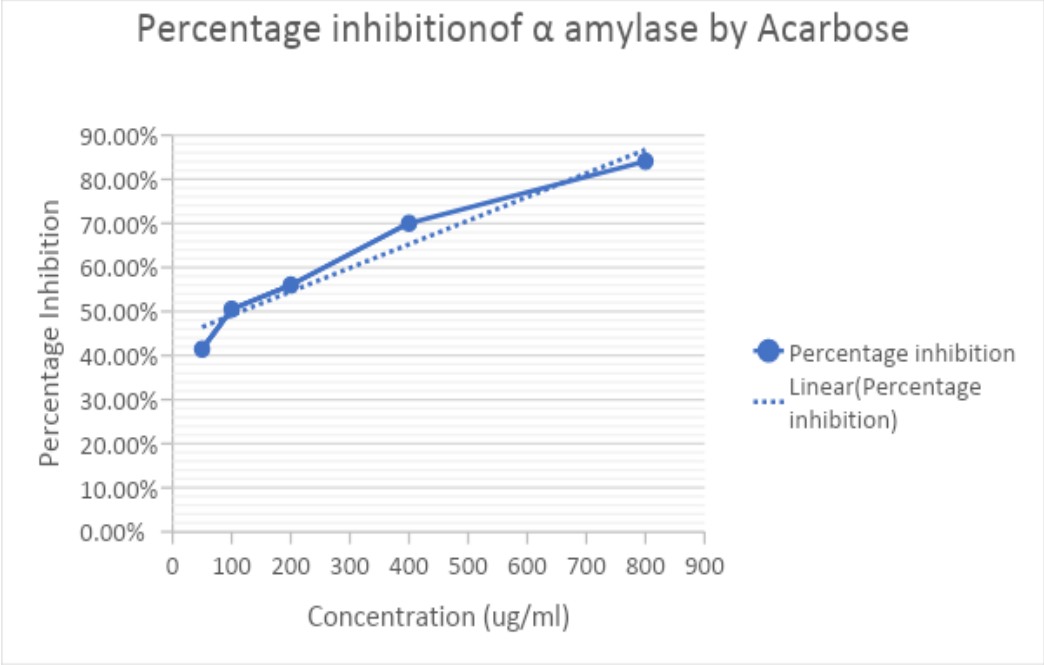


**Fig 15: Graph of Percentage inhibition of α amylase by acarbose.**

**Table 11: Percentage inhibition of α Amylase by Pterocarpus marsupium loaded liposomes.**

| Concentration (ug/ml) | Absorbance | Percentage inhibition |
|---|---|---|
| 100 | 1.110 | 21.88% |
| 200 | 0.951 | 33.07% |
| 400 | 0.642 | 54.82% |
| 800 | 0.531 | 62.63% |
| 1000 | 0.410 | 71.14% |

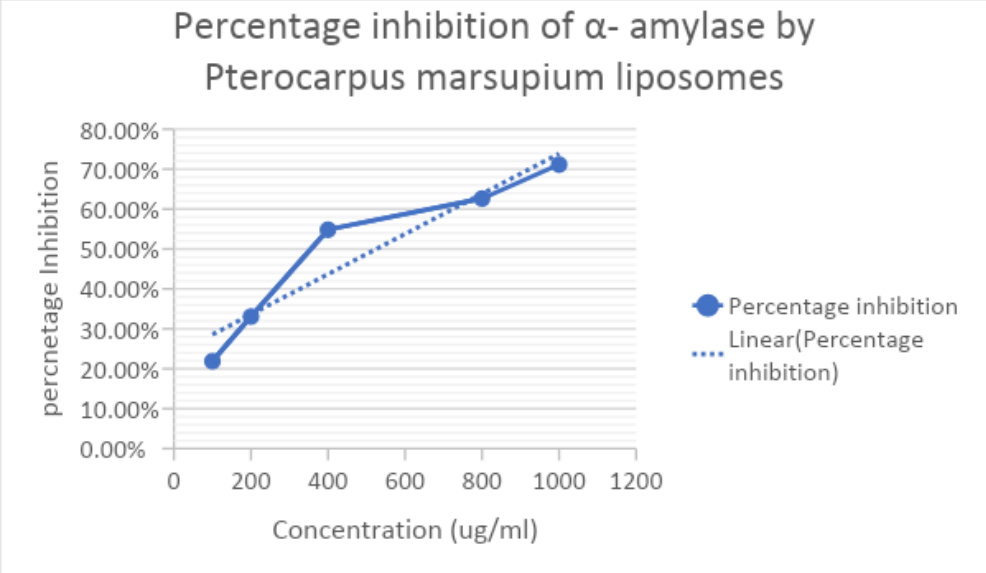


**Fig. 16: Percentage inhibition of α amylase by Pterocarpus marsupium loaded liposomes.**

**Inference:** The IC50 value of positive control was found to be 180 µg/ml and that of biosynthesized *Pterocarpus marsupium* liposomes were 700 µg/ml. The percentage inhibition of Acarbose and *Pterocarpus marsupium* liposomes at lower and higher concentration was found to be 41.44% and 84.09% for positive control Acarbose and 21.88% and 71.14% respectively. Which was similar to the study performed on the Acarbose and *Pterocarpus marsupium* extract.

**Evaluation of Formulated Liposome's Against Diabetes**

**Animal Study (In-vivo study):** The role of phytomedicine in the treatment of diabetes Phytomedicine is once again being investigated for the treatment of diabetes. In therapeutic methodology, many conventional medicines have been derived from prototypic molecules. For each experiment, a certain number of rats were chosen and divided into four groups: A, B, C, and D. Rats in group A were given saline, rats in group B were kept in the Standard

group (oral metformin), and rats in groups C and D were given test drug batches to equate their blood sugar levels to those in groups B, C, and D.

**Effect of test drug on blood sugar level in normal rats:** For the experiment, twenty-four rats of either sex weighing 200-600 gm were chosen. They were divided into four groups of six rats each: A, B, C, and D. Using a 1 ml syringe, blood samples from each rat's tail vein were obtained in a fluoride oxalate bottle. The rats in group 'B' were given a 5 % aqueous solution of the Standard drug orally through a gastric tube at a dosage of 50 mg/100gm body weight per day for 7 days.

A 5% aqueous solution of ***Pterocarpus marsupium*** **liposomes** were prepared and administered orally to rats in groups C and D at a dosage of 50 mg/100 gm body weight per day for 7 days. The rats in group 'A' were used as the control group, with saline water (1 ml/100 gm body) as the treatment. During this time, all animals are fed a standard laboratory diet. On the first, third, fifth, and seventh days of the above drug therapy, blood samples were taken from all rat.

**Effect of test drug on blood sugar level in Alloxan induced hyperglycaemic Rats**

For this analysis, 24 rats of either sex weighing 200-600 gm were chosen. They had to fast for 24 hours prior to the experiment, but water was allowed during that period. Starting with blood, a sample of each rat was calm, as previously mentioned. All of the rats were given a 150 mg/kg body weight intra-peritoneal injection of a freshly prepared 5% aqueous solution of Alloxan tetrahydrate. At the end of the six-hour cycle, all of the rats were given a 5 % glucose solution orally at a dosage of 5 gm/ kg body weight to avoid the Alloxan-induced hypoglycemia. Blood samples were obtained from all of the rats the next day after 24 hours of Alloxan administration, and the rats were classified into four groups: A, B, C, and D, each with six rats. The rats in group B were given a 5% aqueous solution of the standard medication orally in a dosage of 50 mg/100 gm body weight daily for 7 days. Pterocarpus marsupium liposomes in a 5% aqueous solution were prepared and given orally to rats in groups C and D at a dose of 50 mg/100 gm body weight per day for 7 days.

**ANIMAL STUDY (IN-VIVO STUDY)**

**Without inducing diabetes**

**Table 12: Mean Data with standard deviation of Animal study without inducing diabetes.**

| Days | Normal control | Standard | Test 1 in PB | Test 2 in PBS |
|---|---|---|---|---|
| On 1st day of drug treatment | 78.61905±0.51343 | 78.52381±0.44855 | 77.47619±0.39382 | 77.61905±0.4397 |
| On 3rd day of drug treatment | 79.04762±0.51343 | 78.61905±0.44855 | 78.07143±0.39382 | 78.92857±0.4397 |
| On 5th day of drug treatment | 79.71429±0.51343 | 79.33333±0.44855 | 78.04762±0.39382 | 77.69048±0.4397 |
| On 7th day of the drug treatment | 79.61905±0.51343 | 79.35714±0.44855 | 78.42857±0.39382 | 79.52381±0.4397 |

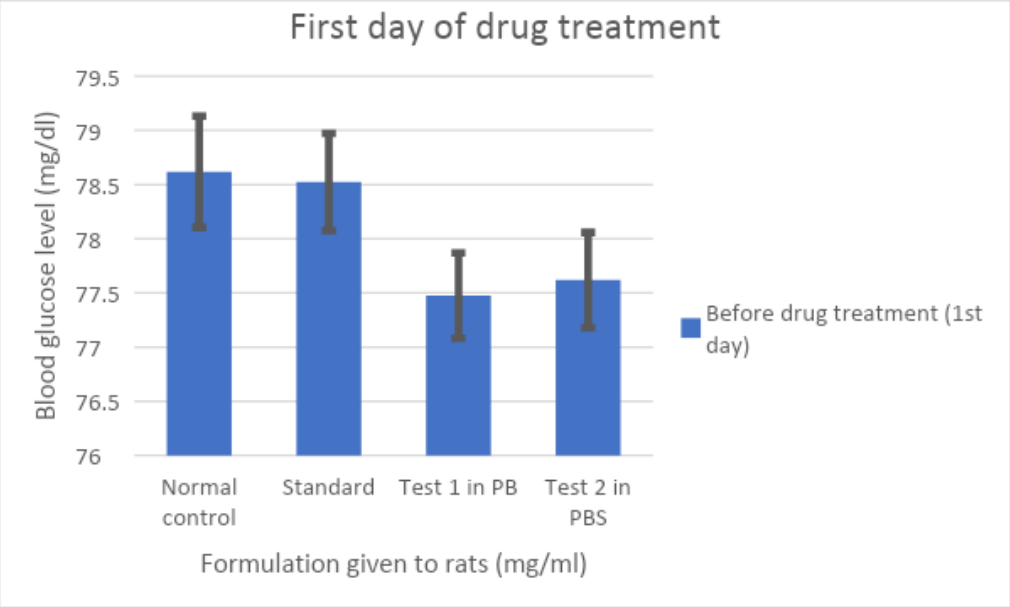


**Fig. 17: Mean data chart of all formulation on first day of drug treatment in rats with SD without inducing diabetes.**

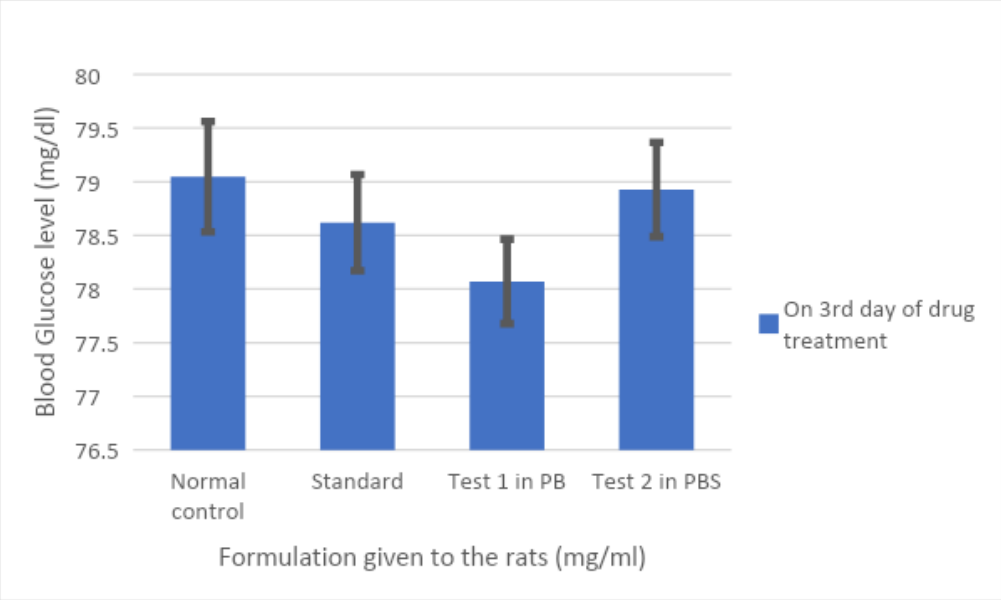


**Fig. 18: Mean data chart of all formulations on 3rd day of treatment in rats with SD without inducing diabetes.**

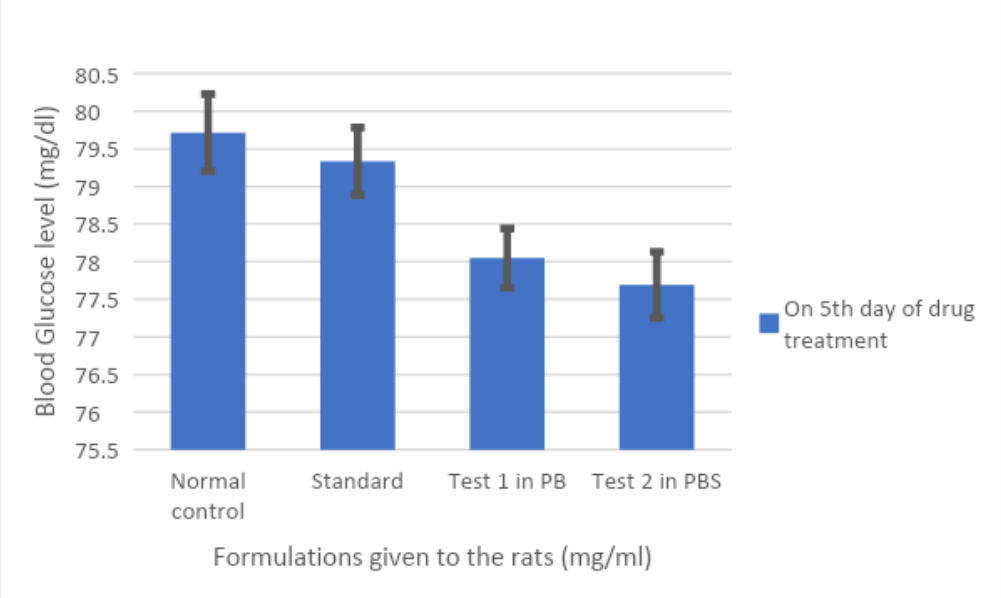


**Fig. 19: Mean data chart of all formulations on 5th day of drug treatment in rats with SD without inducing diabetes.**

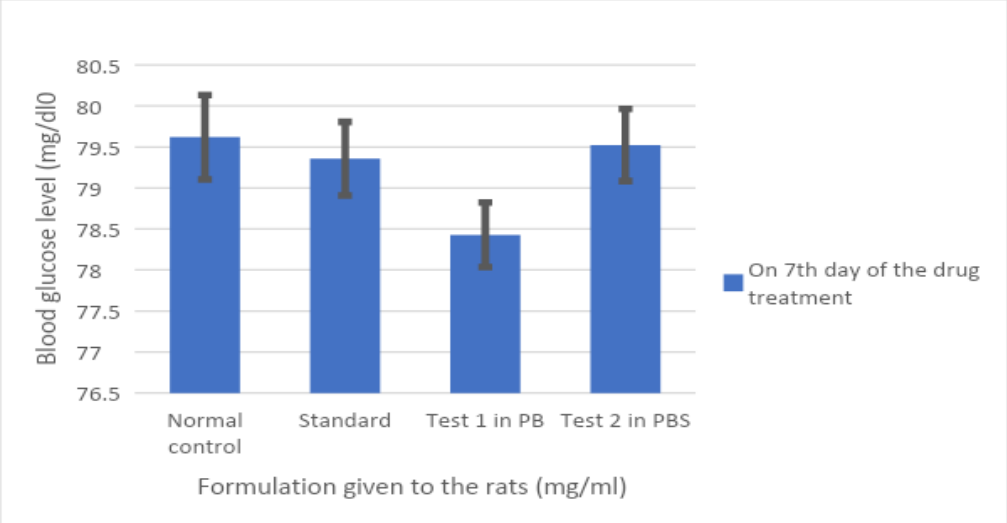


**Fig. 20: Mean data chart of all formulations on 7th day of drug treatment in rats with SD without inducing diabetes.**

**With inducing diabetes**

**Table 13: Mean Data with standard deviation of Animal study with inducing diabetes.**

| Days | Normal control | Standard | Test 1 in PB | Test 2 in PBS |
|---|---|---|---|---|
| After 24hrs giving Alloxan (1st day) | 310.07±1.0126935 | 229.79±1.02263 | 233.12±1.015442 | 232.88±1.013246 |
| On 3rd day of drug treatment | 330.07±1.0126935 | 167.21±1.02263 | 193.12±1.015442 | 192.88±1.013246 |
| On 5th day of drug treatment | 340.31±1.0126935 | 126.69±1.02263 | 132.17±1.015442 | 132.88±1.013246 |
| On 7th day of the drug treatment | 390.07±1.0126935 | 109.55±1.02263 | 113.12±1.015442 | 112.88±1.013246 |

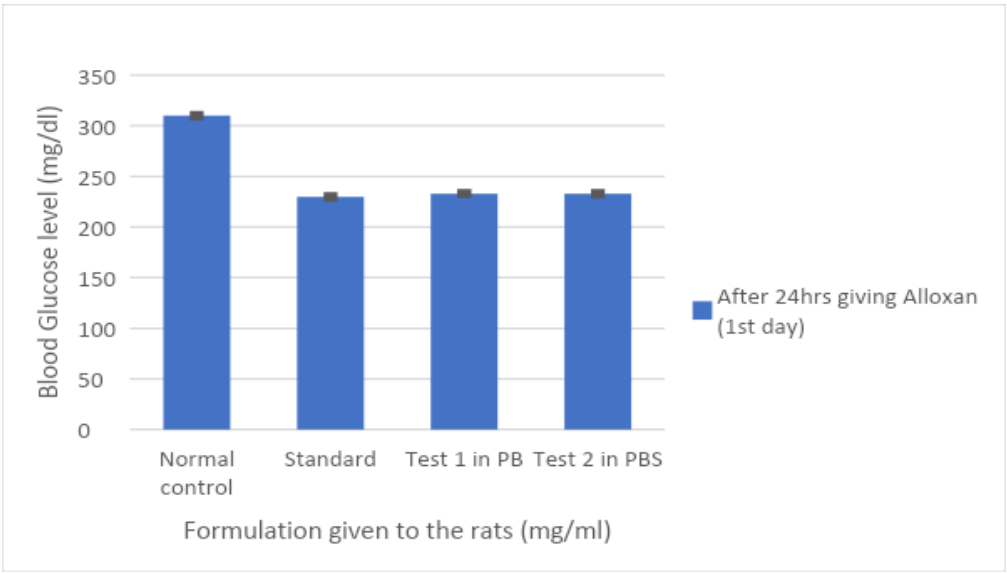


**Fig. 21: Mean data chart of all formulations After 24 hrs giving Alloxan drug treatment (1st day) in rats with SD with inducing diabetes.**

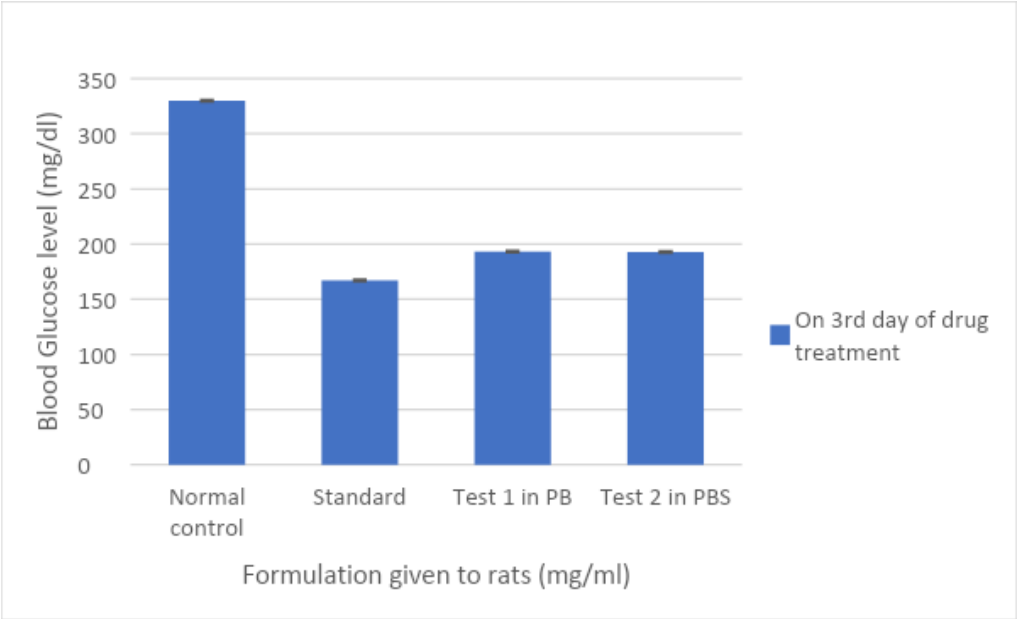


**Fig. 22: Mean data chart of all formulations on 3rd day of drug treatment in rats with SD with inducing diabetes.**

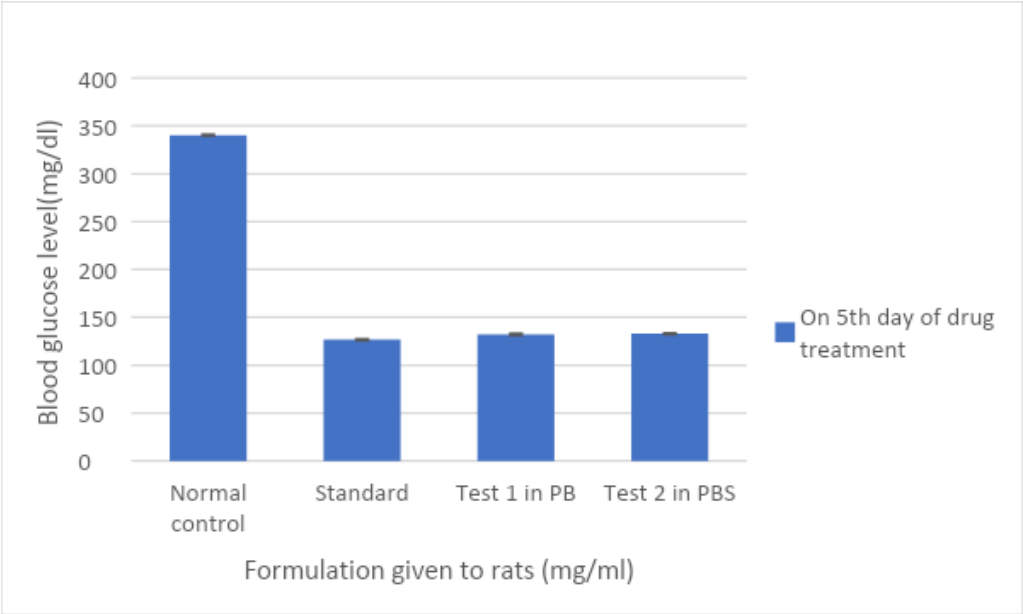


**Fig. 23: Mean data chart of all formulations on 5th day of drug treatment in rats with SD with inducing diabetes.**

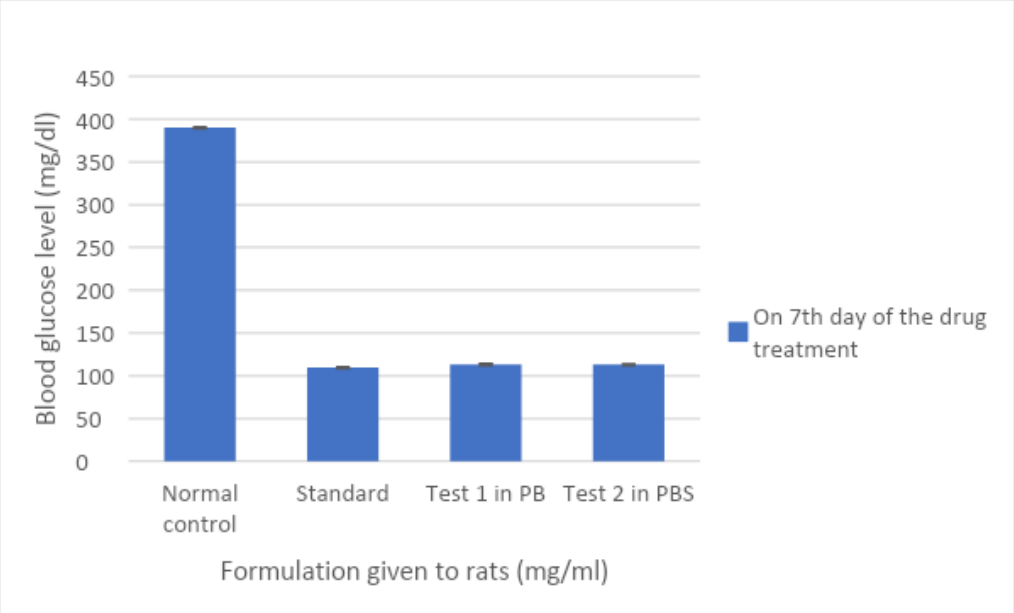


**Fig. 24: Mean data chart of all formulations on 7th day of drug treatment in rats with SD with inducing diabetes.**

**Inference:** In the present study, glucose level was reduced by the experimental drug in swiss albino rats from 3rd day and the Student t-test was also showing the significant difference while comparing with control and alloxan induced swiss albino rats. Hence the drug Vijaysar (*Pterocarpus marsupium*) was having antidiabetic effect. The present study claims that test drugs liposomes of extract of Vijaysar was shown more effective than the normal extract of vijayasar and also showing the significant hypoglycemic effect in diabetic rats (alloxan induced). While there was no increase and decrease the blood sugar level in normal rats.

## CONCLUSION

In this study the liposomes of the *Pterocarpus marsupium* shows good results with comparison the synthetic antidiabetic drugs. It shows the good permeability, stability, in-Vitro Inhibition of the alpha- amylase with reference of acarbose and Zeta sizer and microscopic images shows the wide range of the vesicle size upto um- nm range.

In this present study the effectiveness of the *Pterocarpus marsupium* extract loaded liposomes were more effective than the extract of the *Pterocarpus marsupium* themselves because due to the size reduction and the having the about neutral potential the ability of the drug absorption of a hydrophilic drug through the membrane will be enhanced and the also increases the onset of action and also delay the frequency of the dosing in a day in rats but due to the cholesterol incorporation about 0.1% of the formulation by acid degradation. It can

be minimized by using the DOPC and DPPC type of the polymers which protect the formulation from the degradation by acidic environment of stomach. The effectiveness of the liposomes of vijayasar extract from vijayasar extract was compared by using the data comparision which was mentioned in the article Anti-diabetic investigation of aqueous extract of *Pterocarpus marsupium* (vijayasar(Parmar, Singh, Gupta, Pathak, & Nayak, 2016). According to this article on the $7^{th}$ day of the study the *Pterocarpus marsupium* minimized the blood glucose level up to 280.83 mg/dl but the liposomes of the *Pterocarpus marsupium* minimized the blood glucose level up to 113.12 and 112.88 mg/dl which was near about the normal blood glucose level of the rats 70-110 mg/dl. So, it can say that liposomal formulation of the *Pterocarpus marsupium* was more effective than the normal extract of the *Pterocarpus marsupium*.

## REFERENCES


1. Devgun, M., Nanda, A., & Ansari, S. Pterocarpus marsupium Roxb.-A comprehensive review. *Pharmacognosy Reviews*, 2009; *3*(6): 359.
2. Gairola, S., Gupta, V., Singh, B., Maithani, M., & Bansal, P. Phytochemistry and pharmacological activities of Pterocarpus marsupium: a review. *Int Res J Pharm*, 2010; *1*(1): 100-104.
3. Handa, S., Singh, R., Maurya, R., Satti, N., Suri, K., & Suri, O. Pterocarposide, an isoaurone C-glucoside from Pterocarpus marsupium. *Tetrahedron Letters*, 2000; *41*(10): 1579-1581.
4. Maurya, R., Singh, R., Deepak, M., Handa, S., Yadav, P. P., & Mishra, P. K. Constituents of Pterocarpus marsupium: an ayurvedic crude drug. *Phytochemistry*, 2004; *65*(7): 915-920.
5. Parmar, K. S., Singh, G. K., Gupta, G. P., Pathak, T., & Nayak, S. Evaluation of De Ritis ratio in liver-associated diseases. *International Journal of Medical Science and Public Health*, 2016; *5*(9): 1783.
6. Salih, S. I., Karem, K. K., & Jassim, W. K. An Evaluation of Hemoglobin Concentration in Patients with Diabetes Mellitus Type 2. *International Journal of Drug Delivery Technology*, 2019; *9*(02): 177-181.