Available online on 15.12.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Toxicity and anti-diabetic effectiveness of polymeric nanoparticles containing natural compounds

Trilochan Satapathy[1]*, Abinash Satapathy[2], Neha Yadav[2], Ansuman Satapathy[3], Kunal Chandrakar[4], Shiv Kumar Bhardwaj[1]

1 Columbia Institute of Pharmacy Tekari, Near Vidhansabha, C.G., India

2 College of Veterinary Science and Animal Husbandry, Anjora-491001, Durg, C.G., India

3 Faculty of Pharmacy, Kalinga University, Kotni, Near Mantralaya Naya Raipur – 492101, Chhattisgarh, India

4 University College of Pharmacy, CSVTU, Bhilai, Durg, C.G. India

Article Info:

Article History:

Received 22 Sep 2023
Reviewed 26 Oct 2023
Accepted 19 Nov 2023
Published 15 Dec 2023

Cite this article as:

Satapathy T, Satapathy A, Yadav N, Satapathy A, Chandrakar K, Bhardwaj SK, Toxicity and anti-diabetic effectiveness of polymeric nanoparticles containing natural compounds, Journal of Drug Delivery and Therapeutics. 2023; 13(12):113-124

DOI: http://dx.doi.org/10.22270/jddt.v13i12.6089

*Address for Correspondence:

Dr. Trilochan Satapathy, Department of Pharmacology, Columbia Institute of Pharmacy, Tekari, Near Vidhansabha, C.G., India

Abstract

Objective: To develop the nanoparticle formulations, characterization and evaluation of safety and *in vivo* anti diabetic potential by using experimental animal model.

Methods: Different nano formulations such as Cur-nanoparticles, Pip-nanoparticles as well as Cur-Pip dual drug loaded nanoparticles were developed by nano co-precipitation method. The developed formulations were subjected for FT-IR analysis to determine the drug-drug and drug-polymer interaction. The Differential Scanning Colorimetric (DSC) study was carried out to observe the glass transition. Surface topography of nano formulations were carried out by Scanning Electron Microscopy (SEM). X-ray diffraction study was carried out to determine the crystalline properties of different formulations. Particle size of the polymeric nanoparticles was evaluated by Zeta sizer of nanoseries. Toxicity of nanoformulation was evaluated as per OECD guideline-407. For evaluation of therapeutic effectiveness, *in-vivo* anti-diabetic potential of nano formulations, the Streptozotocin (STZ) induced diabetes model was considered.

Results: The developed formulations were spherical in shape and smooth surfaced. There were no interaction between the drugs and polymers. The result of toxicity study revealed that, there were no changes in behavior, food intake; hematology as well as biochemical parameters were observed that indicates the developed formulation is completely safe. The anti-diabetic effect of different formulations was screened against Streptozotocin induced diabetes in experimental animals. All the formulations were proved as effective in restoring blood glucose level however, Cu+Pi NP (184.15) group showed highest anti diabetic activity in comparison to control group (207.93)

Conclusions: From this study, it was observed that, Curcumin-Piperine dual drug loaded nanoparticles exhibit better anti diabetic potential in comparison to control and Curcumin-NP treated group.

Keywords: Nanoparticles, Streptozotocin, anti-diabetes, toxicity, hematology, biochemical

INTRODUCTION

Diabetes Mellitus (DM) is considered as a metabolic disorder which is characterized by hyperglycemia and the incidence of the disease is high throughout the world. This disorder is common, chronic and serious believed to be due to resulting from insufficient or inefficient insulin or both. The prevalence of DM is rapidly increasing all over the globe at alarming rate. According to the data available with International Federation of Diabetes (IFD), 415 million adults over the world are presently suffering from diabetes, and this value will reach around 642 million by 2040.[1-2] As per the data published in 2018, the overall diabetes burden estimates for the 1·3 billion in population of India mask wide variations across the states of the country, many of which are comparable to large countries in terms of population.[3] This disorder is related to high risk of several complications which is major incumbrance for patients, society, health care systems and economy of the country. The present treatments have limitations due to their side effects, particularly weight gain and hypoglycemia, or contraindications that limit their use.[1] Recent epidemiological data suggested that, the various types of anti-diabetic therapies on long term use can enhance the risk to establish pancreatic cancer. Among the various treatment options available, sulfonylurea, thiazolidinediones, and alpha-glucosidase inhibitors appear to have little or no effect for the establishment of pancreatic cancer where as some of the newer anti diabetic agents are burdened with some concerns in the respect of pancreatic cancer [4-5]. The DPP-IV inhibitor sitagliptine was seen to induce pancreatic ductal metaplasia, and a 4-fold increase in duct cell proliferation [6]. Herbal based medicines are in practice since as of mankind and during many decades chemical evaluations as well as pharmacological screenings have been progressed using many plant extracts to establish their chemical composition as well as to confirm their indications in different health conditions.

Various herbal drugs and herbal extracts, although they are showing great potential and better in-*vitro* findings but demonstrate little or no *in-vivo* actions which may be due to their poor lipid solubility or in appropriate molecular size or both. Hence, poor absorption that in turn ultimately resulting in poor bio-availability. [7] Curcumin (1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-hepadiene, 3, 5,-dione) is one of the several components in the rhizome of turmeric and has been used over centuries in traditional remedies for the treatment of a variety of diseases, including inflammatory conditions.[8] Numerous molecular targets for Curcumin have been identified over the years. Several pre-clinical and clinical studies indicated that, Curcumin is safe at very high concentrations. [9] In spite of its safety and effectiveness, Curcumin is not approved as a therapeutic agent till yet. The major barrier for the clinical use of Curcumin includes is poor oral bioavailability. However, this is mainly due to its poor aqueous solubility, increased intestinal metabolism and hepatic metabolism as well as rapid systemic clearance.[10-11] Now-a-days according to the advancement of technology, novel drug delivery systems paved the way towards the enhancement of bioavailability of many herbal drug delivery systems. These limitations may be overcome by formulating as nanoparticle-based drug delivery system. Many herbal compounds such as Piperine, quercetin, genistein, sinomenine, glycyrrhizin, naringin and nitrile glycoside have proved their capability to enhance the bio-availability when co-administered with other agents. [12] Hence, the primary aim of this study was to develop Curcumin-Piperine nanoparticles by the suitable method and to evaluate the therapeutic effectiveness of prepared formulation in Streptozotocin induced diabetes model.

MATERIALS AND METHODS

Materials

Chitosan (Hi-media, Mumbai, India), Polycaprolactone and Streptozotocin (Sigma-Aldrich, Mumbai, India), Curcumin and Piperine (Sun pure Extract Pvt. Ltd., India) and all other chemicals used in this study were analytical reagent grade.

Preparation of polymeric nanoparticles[13, 14, 15]

In this research, Curcumin/Piperine loaded Chitosan/Polycaprolactone (PCL) nanoparticles were developed by nano-co-precipitation method. Briefly, accurately weighed quantity of pure Curcumin/Piperine and polymer Chitosan and co-polymer PCL were co-dissolved into 10 ml of 90% acetic acid solution to form a homogeneous solution. This solution was then added drop wise under continuous stirring, into 50 ml of distilled water solution to obtain the Curcumin/Piperine loaded Chitosan/PCL nanoparticles. Finally, the obtained nanoparticles were centrifuged at 13,000 rpm/min for 30 min, discarding the supernatant, and re-suspended with 10 ml of distilled water solution for the further characterization and applications.

Spectral analysis (FT-IR) [16]

FTIR stands for Fourier transform infrared which is considered as most useful method for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture and for the analysis of solids, liquids, and gases. The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a development in the manner in which the data is collected and converted from an interference pattern to a spectrum. It is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. The basic principles of FTIR are that, the Molecular bonds vibrate at various frequencies depending on the elements and the type of bonds. For any given bond, there are several specific frequencies at which it can vibrate. In this research, FTIR spectra of pure Curcumin, Piperine, Polycaprolactone (PCL), Chitosan, and finally developed formulations (nanoparticles) were recorded on FTIR spectrometer (Nicolet, model Magna 550, USA). In brief, about 2-3mg of sample was mixed with dried potassium bromide (KBr) of equal weight and compressed under a hydraulic pressure of 600 kg to form a KBr disk. These disks containing samples were scanned from 400 to 4000 cm-1.

Morphological analysis of the Nano formulations by Scanning Electron Microscopy (SEM)

The prepared nanoparticles were subjected to morphological analysis. The surfaces were studied using Scanning Electron Microscope (JEOL.JSM-6480LV, Japan). The nanoparticles were mounted on metal grids using double sided tape and gold coated under vacuum and the image was taken.

Particle size measurement of different nanoparticle formulations

Particle size and poly dispersity index of formulated nanoparticles were measured by using a zeta sizer Nanoseries ((Malvern Instruments, Malvern, UK).

Differential Scanning Colorimetry (DSC)

Differential Scanning Colorimetry (DSC) is a thermo dynamical process in which the difference in the amount of heat required to increase the temperature of sample and reference are measured as a function of temperature. The main aim of DSC is studying the phase transitions such as melting, Glass transitions or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity. Differential Scanning Colorimetry (Mettler-Toledo, PerkinElmer, TA Instrument) was performed to characterize the physical state of Curcumin, Piperine, Polycaprolactone (PCL), Chitosan, and final formulation. About 5 mg of sample was weighed, crimped into an aluminum pan and analyzed at a scanning temperature range from 50 to 300°C at a heating rate of 10°C/min.

X-ray diffraction study (XRD)

X-ray diffraction analysis was employed to detect the crystallinity of the pure drug and the nanoparticle formulation, which was conducted using a Philips PW 3710 x-ray diffractometer (XRD) with a copper target and nickel filter (Philips Electronic Inst, Holland). Powders were mounted on aluminum stages with glass bottoms and smoothed to a level surface. The XRD pattern of each sample was measured from 10 to 50 degrees 2-theta using a step increment of 0.1 2-theta degrees and a dwell time of 1 second at each step .

Determination of Drug Entrapment Efficiency

The percentage of drug entrapped in the different nanoparticle formulations was determined by weighing the accurately amount of different nanoparticle formulations and ultracentrifugation at 15000rpm for 45 min in an ultracentrifuge to separate the loaded drugs from the formulations. Then the supernatant was separated and analyzed by U V Visible spectrophotometer at 421nm and 342nm for Curcumin and Piperine respectively. The amount of drug entrapped was calculated by the formula given below.

DEE (%) = (Actual drug content/Theoretical drug content) x100

In-vitro Release Study

Curcumin nanoparticles, Piperine nanoparticles and nanoparticles containing both the drugs were prepared by

nano co-precipitation method. *In Vitro* drug release from different nano particle formulations were performed by using membrane dialysis method. It is one of the most popular and versatile method to assess the drug release pattern of different nano sized dosage forms. At first the membrane was clamped in open glass tube for drug release. A 200ml of Phosphate buffer solution (PBS) with pH 7.4 and other with pH 3.4 were used as dissolution medium and taken in a receiver compartment. Before the release test, an accurately weighed quantity of the formulation dissolved in accurate quantity of the dissolution medium and placed in the glass tube maintained with temperature 37^{o}C at 100 rpm by magnetic stirrer and bead. A 5ml of aliquot was withdrawn from the receiver compartment at fixed time interval and maintained the sink condition with fresh medium immediately. Samples were analyzed by UV-Visible spectrophotometer at 421 and 342 for Curcumin and Piperine respectively.

IN VIVO STUDY:

The developed formulations were then screened for *In vivo* pharmacological activity to evaluate their toxicity, therapeutic effectiveness against STZ induced diabetes using rat model.

Experimental animals:

To carry out the toxicity study, Wistar albino rats were used because rats are recommended rodent species for conducting acute toxicity studies as per OECD guideline and also sensitive for expression of toxic responses. All the experimental animals were obtained from Animal house facility, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India. Animals were maintained at standard environmental temperature and humidity, fed commercial pellet rat chow (M/s Hindustan lever foods, Bangalore, India) and water *ad libitum*. A 12:12h light and dark cycle (7am-7pm) was maintained throughout the experimental protocol. This study was conducted with prior permission to Institutional Animal Ethics Committee, Columbia Institute of Pharmacy, Raipur, Chhattisgarh, India, Regd. No (1321/po/ReBi/S/10/CPCSEA dated 22.10.2014).The animals were allowed to acclimatize to laboratory conditions two weeks prior to the experiments.

Grouping of animals:

In both the experiments, the animals were randomly allocated into different groups. The grouping of animals with their numberings and treatment schedule for toxicity study is depicted in Table-3 whereas for STZ induced diabetes model, the grouping and treatment details are presented in table-13. The preliminary experiment was conducted for evaluation of toxicity of developed formulations. In this experiment, ten healthy animals of controlled age and body weight in each group were used (Five males and five females) as per Organization of Economic Co-operation and Development (OECD-407) guideline. The therapeutic effectiveness of the developed formulations was investigated using STZ induced diabetes rat model. For this experiment six animals in each group were used. All the female animals used in these experiments were nulliparous and non-pregnant. The animals were housed five each, of same gender in polypropylene cages provided with bedding of husk. Each animal was fur markedwith picric acid.

Selection of dose and route of administration:

All the dosing was continued up to 28 days .The animals were observed daily for any toxic manifestation and mortality. Body weights and food consumption were measured at different time interval during the experimental period. The oral route was selected for administration of test substances because oral route is considered as a proposed therapeutic route of administration in human being.

Repeated dose toxicity study (OECD-407, 2008)[17]

Repeated dose toxicity was performed using Wistar rats of either gender of controlled age and body weight as per OECD guideline-407.Animals were randomly allocated into five groups (Five male and five female in each group).All the animals received treatments after overnight fasting. The details of animal grouping, their numbers and treatment are described in table no-3 below.

Table 1: Allocation of experimental animals into different groups for evaluation of toxicity of different nano formulations.

Group No	Treatment	Gender	Animal Numbers
I	Control(Vehicle)	Male	1-5
		Female	6-10
II	Blank nanoparticles	Male	11-15
		Female	16-20
III	Cu-nanoparticles	Male	21-25
		Female	26-30
IV	Pi-nanoparticles	Male	31-35
		Female	36-40
V	Cu+Pi nanoparticles	Male	41-45
		Female	45-50

All treatments were administered orally suspended in 0.3 %CMC (vehicle)

RESULTS:

Composition and encapsulation efficiency of different nanoformulations

The details of Composition i.e drug polymer ratio and encapsulation efficiency of different nanoformulations has been depicted in table no 2. The encapsulation Efficiency (EE %) was found to be 82%,71% and 67% for Curcumin nanoparticles, Piperine nanoparticles and for Cu+Pi dual drug loaded nanoparticles(NP).

Table-2. Composition and encapsulation efficiency of different nanoformulations.

Composition	Blank nanoparticles	Curcumin nanoparticles	Piperine nanoparticles	Cu+Pi NP
Distilled water (ml)	50	50	50	50
Curcumin (mg)	00	100	00	50
Piperine (mg)	00	00	100	50
Chitosan (mg)	50	50	50	50
PCL (mg)	950	950	950	950
Encapsulation efficiency (%)	-------	82%	71%	67%

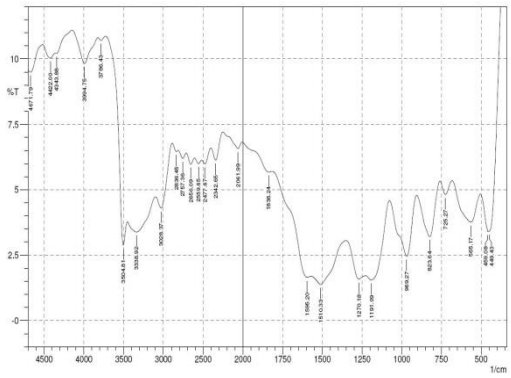

Figure 1: FTIR spectra of of Pure Curcumin

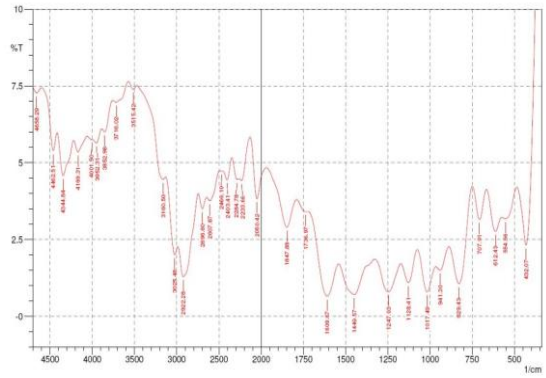

Figure 2: FTIR spectra of Piperine

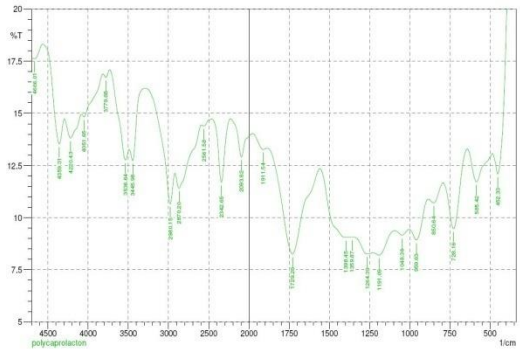

Figure 3: FTIR spectra of Polycaprolactone (PCL)

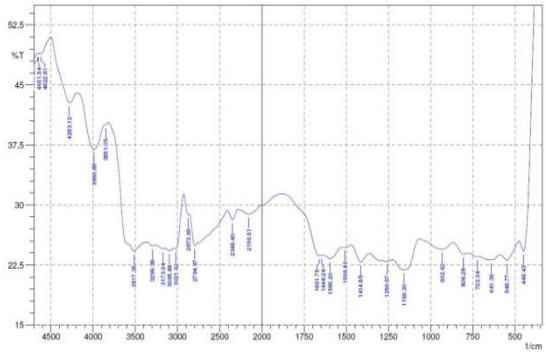

Figure 4: FTIR spectra of Chitosan

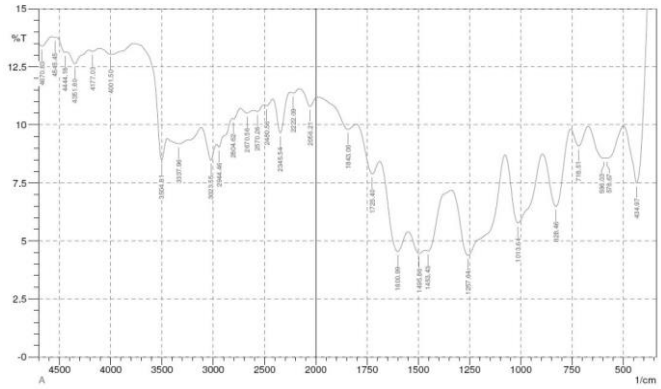

Figure 5: FTIR spectra of Final Formulation

Scanning Electron Micrographs of different Nanoparticle formulations

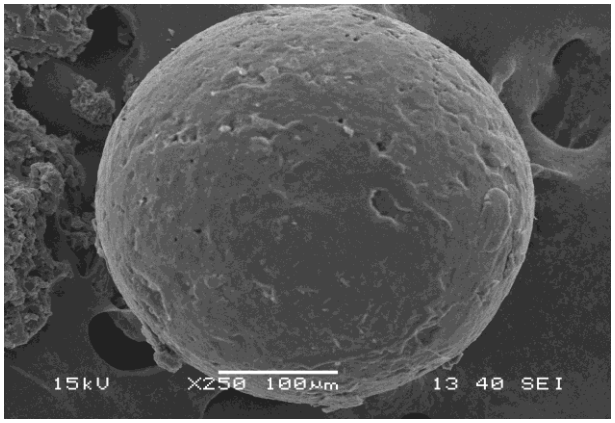

Figure 6: Scanning Electron Micrographs of Blank NP loaded NP

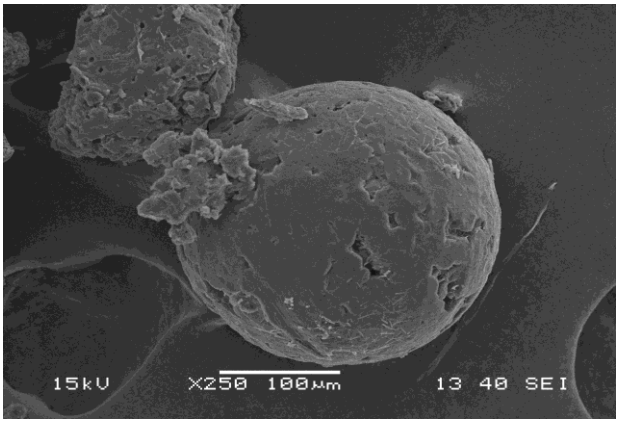

Figure 7: Scanning Electron Micrographs of Curcumin

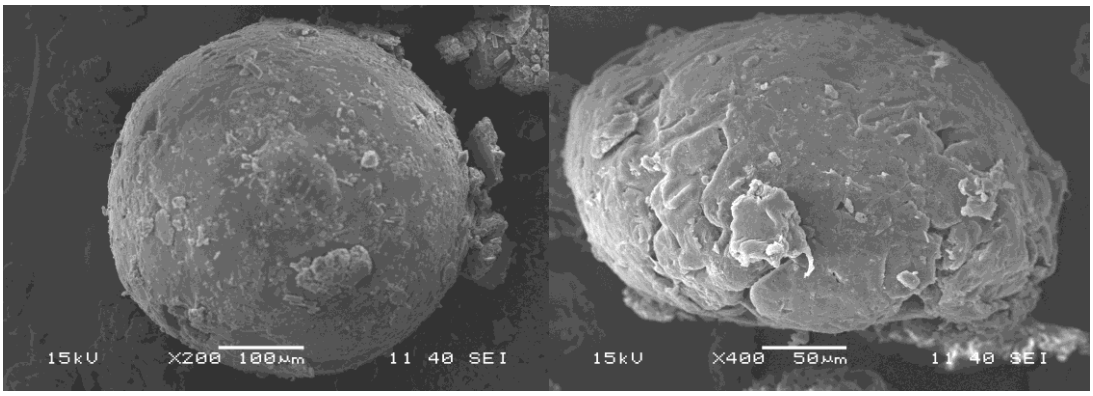

Figure 8: Scanning Electron Micrographs of Piperine loaded NP

Figure 9: Scanning Electron Micrographs of Cu+Pi NP

A Mean particle size and poly dispersity Index of different nano formulations.

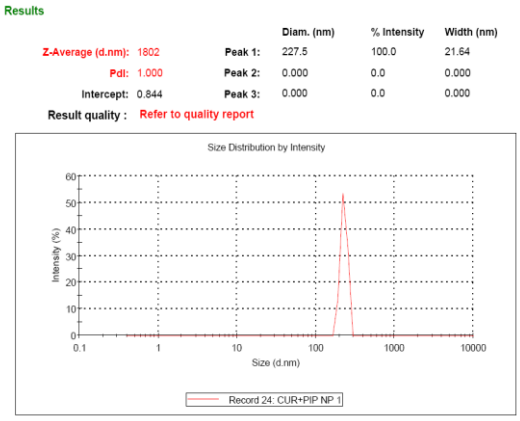

Figure 10: Graph of mean particle size and poly dispersity index of Curcumin NPs

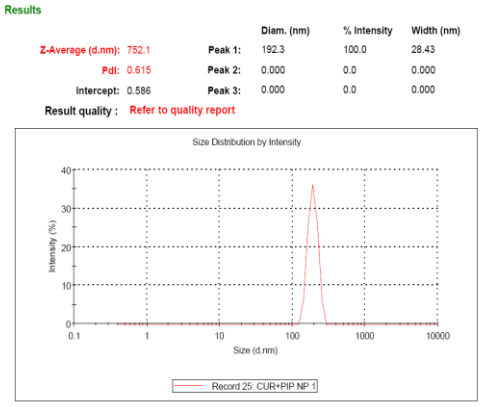

Figure 11: Graph of mean particle size and poly dispersity index of Blank NPs

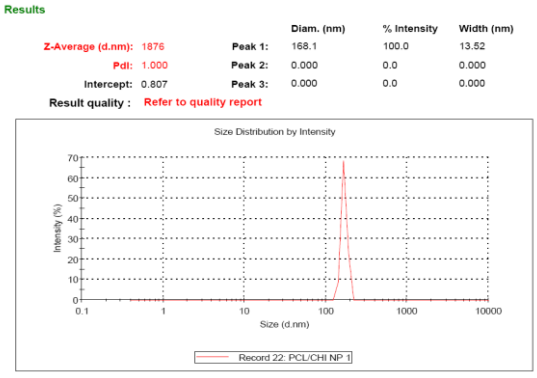

Figure 12: Graph of mean particle size and poly Dispersity index of Cu+Pi Nanoparticles

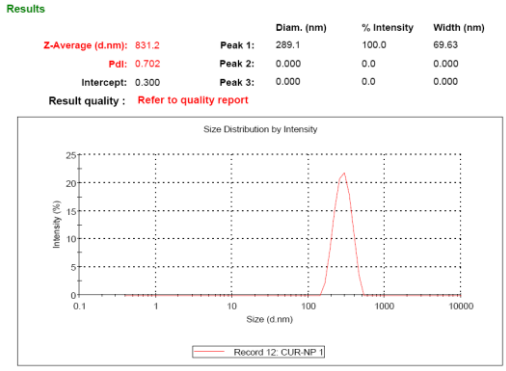

Figure 13: Graph of mean particle size and poly dispersity index of Piperine Nanoparticles

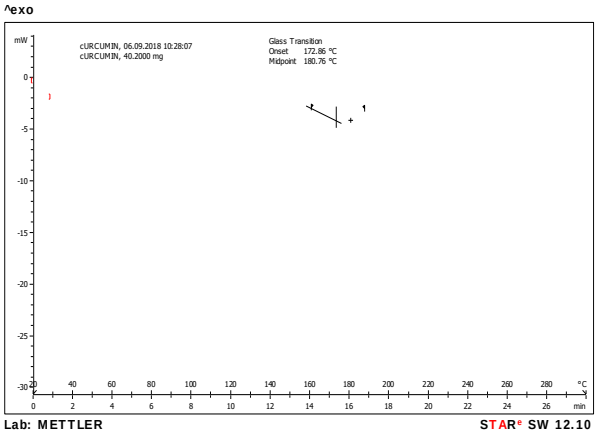

Figure 14: Differential scanning Colorimetric spectra of Curcumin

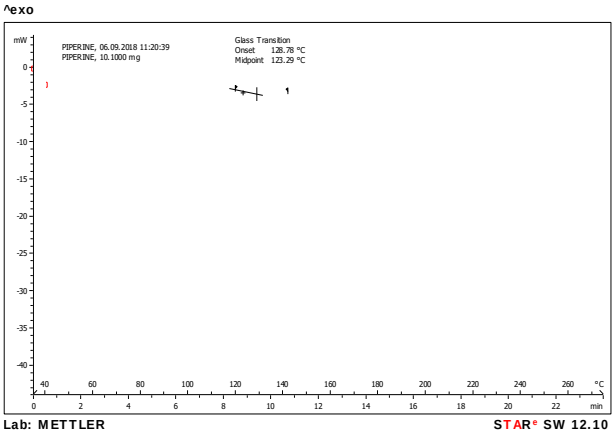

Figure 15: Differential scanning Colorimetric spectra of Piperine

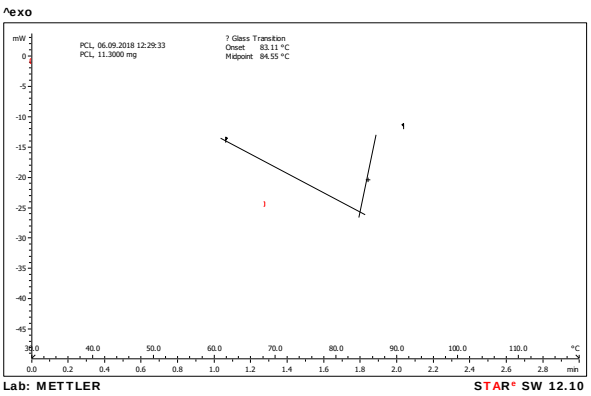

Figure 16: Differential scanning Colorimetric spectra of Poly caprolactone (PCL)

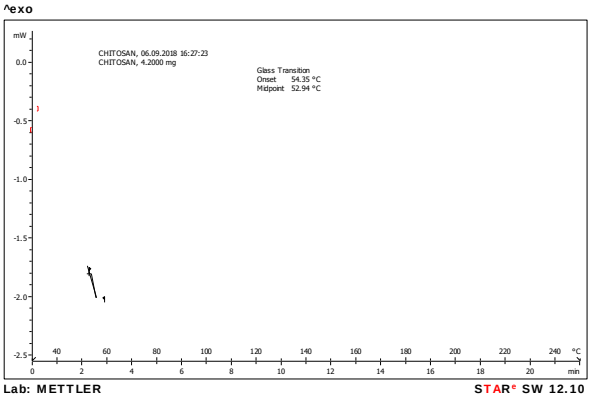

Figure 17: Differential scanning Colorimetric spectra of Chitosan

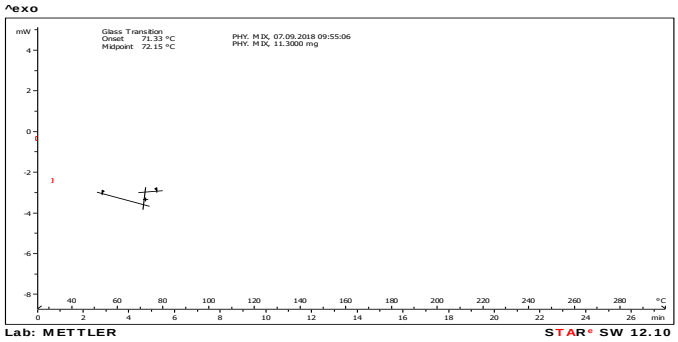

Figure 18: Differential scanning Colorimetric spectra of Final Formulation (Cur+ PIP+ Chitosan/PCL)

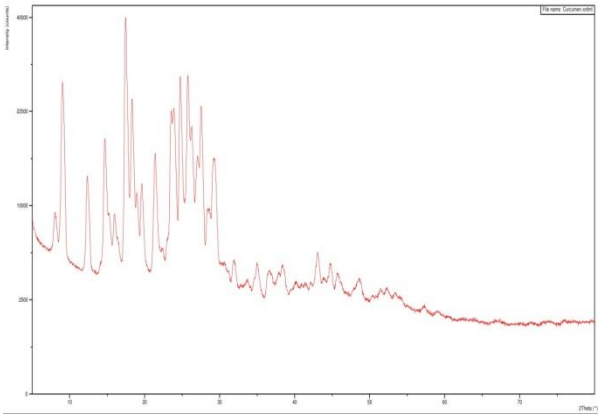

Figure 19: XRD of Curcumin

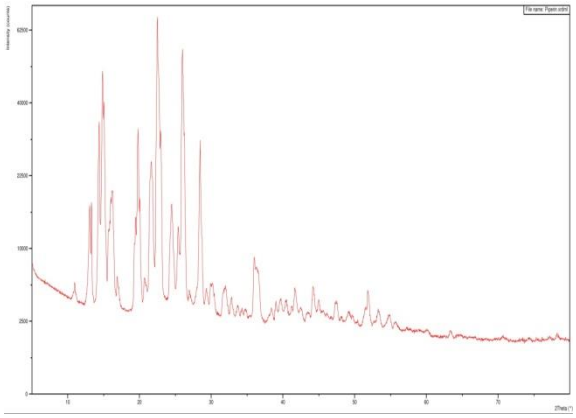

Figure 20: XRD of Piperine

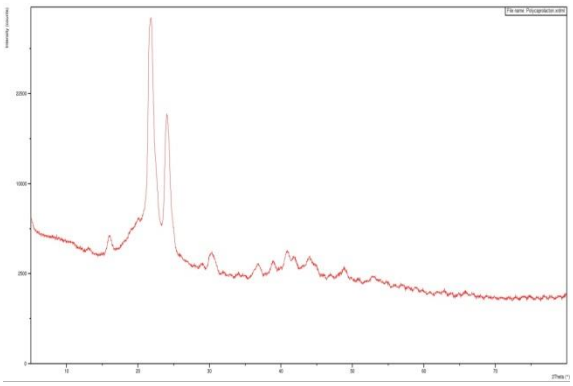

Figure 21: XRD of Polycaprolactone

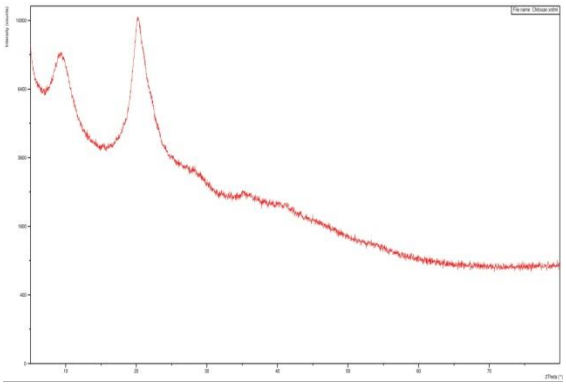

Figure 22: XRD of Chitosan

Table 3: **Summary of Functional Observational Battery (FOB)** Gender-MALE

DAY-AT TERMINATION

Group no	**I**	**V**
Dose	Vehicle	Cu+Pi Nanoparticles
Number of animals Observed	5	5
Number of animals within normal limit	5/5	5/5
Number of animals with significant deviation	0/5	0/5
PARAMETERS EVALUATED		
Alterations home cage	0/5	0/5
Piloerection	0/5	0/5
Reaction to removal	0/5	0/5
Reaction to handling	0/5	0/5
Palpebral closure	0/5	0/5
Lacrimation	0/5	0/5
Salivation	0/5	0/5
Animal appearance	0/5	0/5
Gait	0/5	0/5
Severity of Gait	0/5	0/5
Mobility Score	0/5	0/5
Aurosal	0/5	0/5
Respiration	0/5	0/5
Tonic Movement	0/5	0/5
Clonic movement	0/5	0/5
Stereotype	0/5	0/5
Bizarre behavior	0/5	0/5
Vocalization	0/5	0/5
Rearing	0/5	0/5
Urination	0/5	0/5
Defecation	0/5	0/5
Touch Response	0/5	0/5
Visual response	0/5	0/5
Auditory Response	0/5	0/5
Pupillary Response	0/5	0/5
Tail pinch response	0/5	0/5

Table 4: **Summary of Functional Observational Battery (FOB)** Gender-FEMALE

DAY-AT TERMINATION

Group no	**I**	**V**
Dose	Vehicle	Cu+Pi Nanoparticles
Number of animals Observed	5	5
Number of animals within normal limit	5/5	5/5
Number of animals with significant deviation	0/5	0/5
PARAMETERS EVALUATED		
Alterations home cage	0/5	0/5
Piloerection	0/5	0/5
Reaction to removal	0/5	0/5
Reaction to handling	0/5	0/5
Palpebral closure	0/5	0/5
Lacrimation	0/5	0/5
Salivation	0/5	0/5
Animal appearance	0/5	0/5
Gait	0/5	0/5
Severity of Gait	0/5	0/5
Mobility Score	0/5	0/5
Aurosal	0/5	0/5
Respiration	0/5	0/5
Tonic Movement	0/5	0/5
Clonic movement	0/5	0/5
Stereotype	0/5	0/5
Bizarre behavior	0/5	0/5
Vocalization	0/5	0/5
Rearing	0/5	0/5
Urination	0/5	0/5
Defecation	0/5	0/5
Touch Response	0/5	0/5
Visual response	0/5	0/5
Auditory Response	0/5	0/5
Pupillary Response	0/5	0/5
Tail pinch response	0/5	0/5

Table 5: **Result of different nano formulations on hematological findings in Male Wistar rats for Twenty eight days**

Groups	Hb (g/dl)	Total RBC(10x12 /L)	Rt (%)	HCT (%)	MCV(fl)	MCH (pg)	MCHC (%)	Platelets (10^3/µL)	Total WBC (10^9/L)	Pt. (Sec)
Control	13.95±0.71	7.02±0.70	4±0.96	39.2±1.30	52.4±3.64	20.4±2.07	35.8±4.02	425.6±21.14	9.36±1.30	13.6±2.07
Blank nanoparticle	9.55±1.62	4.82±0.39	2.8±1.04	32.6±3.64	44.8±4.32	14.6±2.88	29.8±2.28	371.6±25.55***	8.36±1.18	9.4±0.89
Curcumin nanoparticles	12.43±1.16	6.5±0.48	3.82±0.84	38.4±1.51	51.4±1.67	18.8±2.77	34±3.67	415.8±19.99	8.14±1.06	11.4±1.51
Piperine nanoparticles	12.15±2.12	6.42±0.65	3.4±0.94	37.6±1.14	49.4±3.13	19.2±2.58	33.8±3.63	421.4±23.22	8.76±0.57	11.8±3.34
Curcumin+Piperine nanoparticle	13.35±1.49	6.82±0.84	3.8±1.01	37.2±3.56	50.4±2.30	19.6±2.07	35±4.79	423.6±21.91	8.96±0.92	12.8±3.27

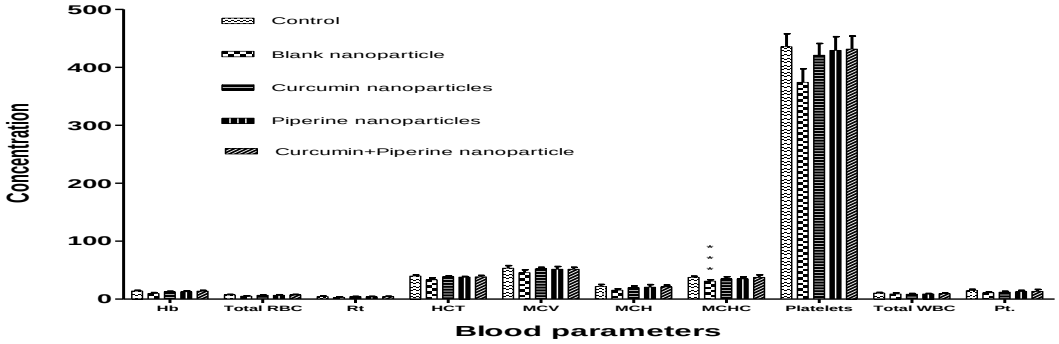

Figure 23: **Effect of different nano formulations on hematological findings in Male rats for Twenty eight days**

Table 6: **Hematological findings of different nano formulations in Female Wistar rats repeated dose toxicity for twenty eight days**

Groups	Hb (g/dl)	Total RBC(10x12 /L)	Rt (%)	HCT (%)	MCV(fl)	MCH (pg)	MCHC (%)	Platelets (10^3/µL)	Total WBC (10^9/L)	Pt. (Sec)
Control	13.95±0.71	7.02±0.70	4±0.96	39.2±1.30	52.4±3.64	20.4±2.07	35.8±4.02	425.6±21.14	9.36±1.30	13.6±2.07
Blank nanoparticle	9.55±1.62	4.82±0.39	2.8±1.04	32.6±3.64	44.8±4.32	14.6±2.88	29.8±2.28	371.6±25.55***	8.36±1.18	9.4±0.89
Curcumin nanoparticles	12.43±1.16	6.5±0.48	3.82±0.84	38.4±1.51	51.4±1.67	18.8±2.77	34±3.67	415.8±19.99	8.14±1.06	11.4±1.51
Piperine nanoparticles	12.15±2.12	6.42±0.65	3.4±0.94	37.6±1.14	49.4±3.13	19.2±2.58	33.8±3.63	421.4±23.22	8.76±0.57	11.8±3.34
Curcumin+Piperine nanoparticle	13.35±1.49	6.82±0.84	3.8±1.01	37.2±3.56	50.4±2.30	19.6±2.07	35±4.79	423.6±21.91	8.96±0.92	12.8±3.27

Data are represented as mean±SD (n =5), significantly different at *p<0.05, **p<0.01, ***p<0.001

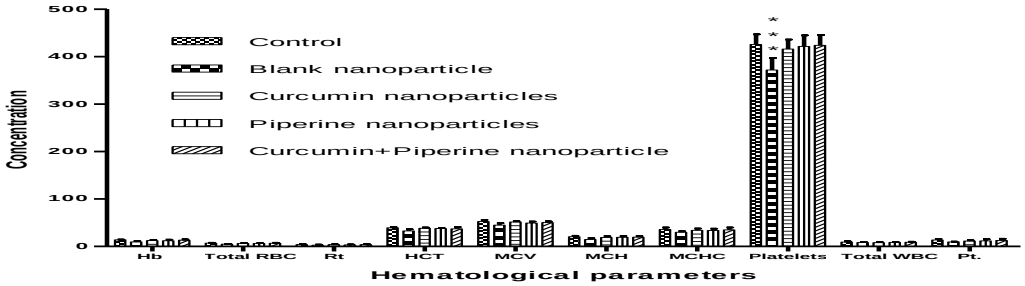

Figure 24: **Hematological findings of different nano formulations in Female Wistar rats repeated dose toxicity for twenty eight days.**

Table 7: **Effect of different formulations on Biochemistry of male rats repeated dose toxicity (Twenty eight days study)**

Gender-Male

Groups	Total protein(g/dl)	BUN (Mg/dl)	ALT (IU/L)	AST (IU/L)	γGT (U/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDH (IU/L)	Bilirubin (mg%)	Albumin (g/dl)
Control	7.14±0.97	30.42±1.30	36.24±4.56	66.2±9.06	14.44±2.55	63.94±8.22	108.06±8.72	324.56±20.40	0.61±0.08	3.57±0.38
Blank nanoparticle	7.54±0.93	36.94±4.01	40.72±1.39	68.44±4.25	18.12±1.08	67.49±3.20	115.3±7.88	361.5±11.15***	0.70±0.03	4.29±0.63
Curcumin nanoparticles	7.42±0.72	35.08±4.07	39.72±1.65	67.22±4.28	17.4±1.29	67.6±2.70	113.6±9.07	357.2±12.51**	0.68±0.05	3.9±0.45
Piperine nanoparticles	7.18±0.61	35.02±4.32	39.56±1.90	67.4±4.39	16.88±1.54	67.64±2.57	114.12±9.80	359.2±9.20**	0.72±0.05	3.79±0.41
Curcumin+Piperine nanoparticle	7.4±1.06	32.32±1.85	37.68±3.05	67.5±7.76	15.52±1.81	65.46±5.73	109.32±9.98	327.2±21.30	0.64±0.05	3.57±0.54

Data are represented as mean±SD (n =5), significantly different at *p<0.05, **p<0.01, ***p<0.001

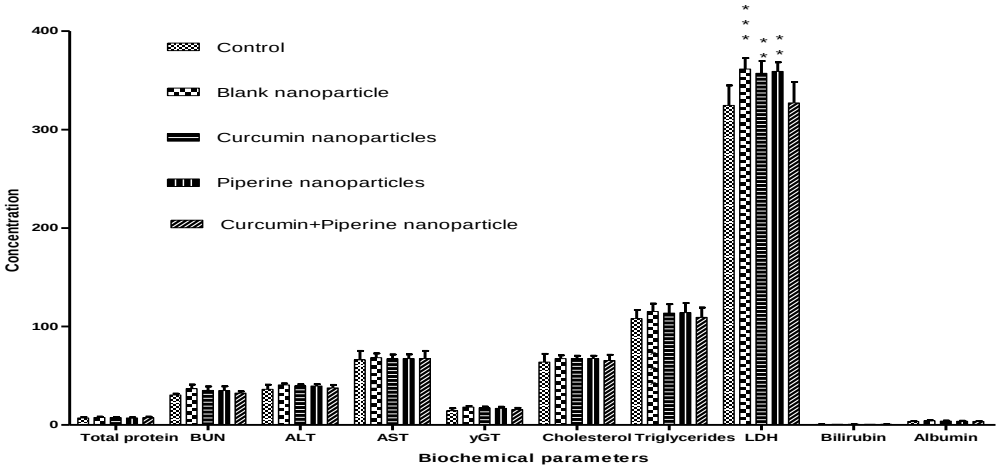

Figure 25: **Effect of different formulations on Biochemistry of male rats repeated dose toxicity (Twenty eight days study).**

Table 8: Effect of different formulations on Biochemistry of female rats repeated dose toxicity (Twenty eight days study).

Groups	Total protein(g/dl)	BUN (mg/dl)	ALT (IU/L)	AST (IU/L)	γGT (U/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDH (IU/L)	Bilirubin (mg%)	Albumin (g/dl)
Control	7.04±1.00	30.48±1.39	36.48±4.15	65.4±7.70	13.8±2.77	63.6±5.54	107.2±9.25	324±20.40	0.61±0.08	3.49±0.35
Blank nanoparticle	7.34±0.88	36.68±3.90	40.44±1.44	68±4.30	17.8±1.09	67±3.16	114.6±7.19	360.6±11.23***	0.69±0.02	4.09±0.69
Curcumin nanoparticles	7.18±0.88	34.88±4.01	39.24±1.98	66.4±4.27	16.4±1.51	66.8±3.11	112.2±8.75	355.6±11.52**	0.67±0.03	3.85±0.44
Piperine nanoparticles	7.06±0.61	34.82±4.28	39.12±2.12	66.4±4.72	16.16±1.62	66.44±3.58	112.32±8.94	356.4±9.28**	0.68±0.03	3.74±0.45
Curcumin+Piperine nanoparticle	7.18±1.04	31.32±1.49	37.08±3.34	66.5±7.77	14.32±2.80	64.46±5.48	108.12±9.26	325±20.40	0.63±0.09	3.494±0.59

Data are represented as mean±SD (n =5), significantly different at *p<0.05, **p<0.01, ***p<0.001

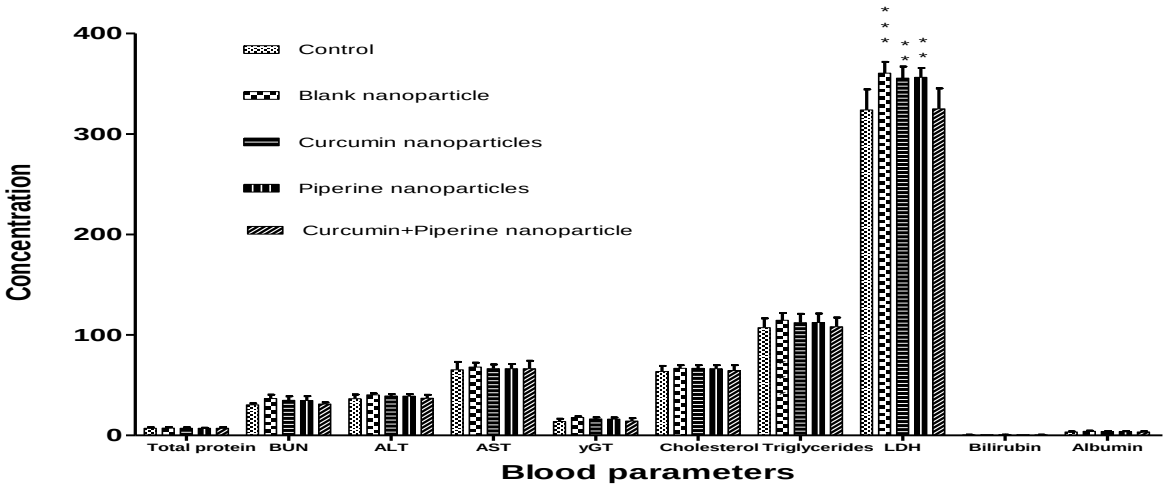

Figure 26: Effect of different formulations on Biochemistry of female rats repeated dose toxicity (Twenty eight days study)

Table 9: Effect of different formulations on anti-diabetic activity against Streptozotocin induced diabetes in experimental animals (Rats)

Groups	0 day	7 day	14 day	21 day	28 day
Control	187.15±1.24	192.78±0.76***	177.42±0.83***	202.06±1.76***	207.93±1.90***
Diabetic control	187.41±0.74	180.82±0.92	198.46±1.70	124.51±0.83	104.92±1.82
Blank nanoparticle	185.64±1.27	175.13±0.97*	157.99±0.99***	180.61±0.60***	183.98±0.99***
Curcumin nanoparticles	187.14±0.90	176.58±0.87	178.06±1.00***	179.04±0.81***	181.30±0.66***
Piperine nanoparticles	186.80±0.88	176.75±0.53	176.91±1.88***	180.84±2.20***	183.15±2.20***
Curcumin+ Piperine nanoparticle	187.63±1.16	177.42±0.64	179.94±1.89***	181.17±2.24***	184.15±2.02***

Data are represented as mean±SD (n =5), significantly different at *p<0.05, **p<0.01, ***p<0.001

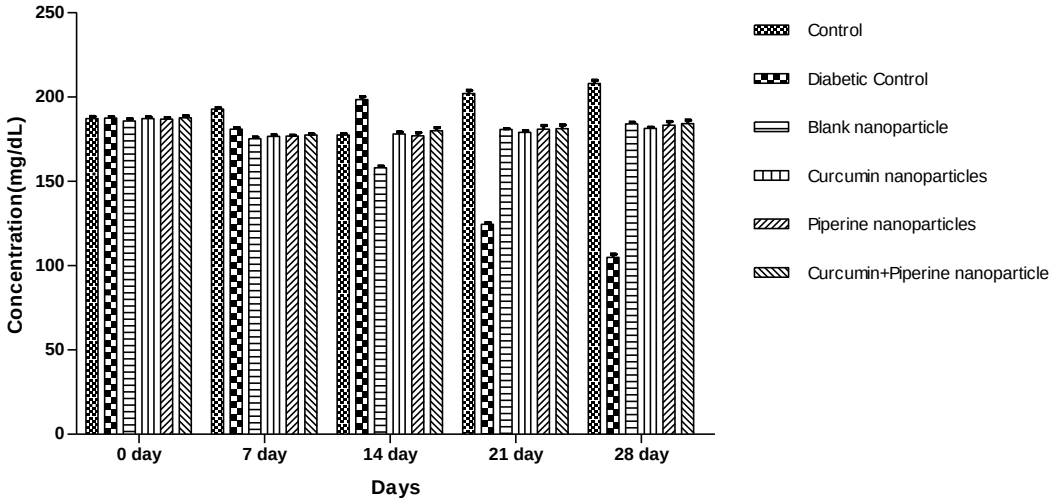

Figure 27: Effect of different formulations on Anti diabetic activity against Streptozotocin induced diabetes in experimental animals (Rats).

DISCUSSION AND CONCLUSION:

The FT-IR spectra of Curcumin, Piperine, PCL, Chitosan and final formulation were depicted in fig 1, fig 2,fig 3, fig 4 and fig 5 respectively. The result of FT-IR spectral analysis indicated that, in the nanoparticle formulation the major characteristic bands of both the drug and polymer are displayed without any significant spectral shift. This indicates that there was no potential interaction between the different components of the final formulation. From the SEM study, our observations indicated that, drugs like Curcumin and piperine both are compatible to polymer and co polymer used during preparation there by nanoparticles of appropriate size, shape and smooth surface have been obtained and there was no significant change in their surface topography. For final formulation the mean particle size fall in the range of 831.2 nm with polydispersity Index value is 0.702. The DSC thermo gram of Curcumin, Piperine, PCL, Chitosan and final formulation (Cur+ Pip+ PCL/Chitosan) have been represented in fig no.14, fig no.15, fig no.16, fig no.17 and fig no.18 respectively. The DSC curve of Curcumin showed a single endothermic peak being started at 172.82°C and ended at

180.76°C. Similarly the DSC curve of piperine also showed a single endothermic peak being started at 128.78°C and ended with midpoint 123.29°C. The DSC thermogram of PCL showed a broad endothermal peak being started at 83.11°C and ended at 84.55°C. The DSC thermogram of Chitosan showed a less intense endothermal peak being started at 54.35°C and ended at midpoint 52.94°C. The DSC thermogram of final formulation showed two major endothermal peaks one being started at 71.33°C and ended at midpoint 72.15°C and other peak at about 114°C -115°C as well as one less intense peak at about 152°C-154°C.Here in the final formulation the sharp endothermic peak of pure Curcumin was shifted to a lower temperature. Such behavior suggests the minute or partial loss of drug crystallinity when the drug was incorporated into the nanoparticle formulation. Since there was no major shift in the glass transition of the polymer in drug loaded formulation, it can be concluded that there was no significant interaction occurring between the drug and polymer. The X-ray diffraction of Curcumin, piperine, Poly caprolactone (PCL), Chitosan have been carried out and are represented in fig.19, fig.20, fig.21, fig.22 respectively. The different formulations were also screened for various *in vitro* parameters before proceed to *in-vivo* activity. The 28 days repeated dose toxicity studies were carried out as per OECD guideline-407. The functional observational battery (FOB) was carried out to quantify their neurotoxic effects. In this experiment the FOB for group-I and group-V animals (comparison study) were carried out to evaluate the effects of developed nano formulations on alteration of neuro behavioral activities in both the gender of rat strain. The result of Functional Observational Battery (FOB), has been indicated in table no-3 and table no-4 for male gender and female gender respectively. The results of FOB in both the gender did reveal neither any abnormalities nor any mortality. The blood withdrawn from retro orbital plexus of different groups of animals and hematological as well as biochemical tests was performed. There were no major changes of in values of test parameters of control and test group of animals that indicates the final formulation did not exhibit any toxicity. The anti-diabetic potential of developed formulations was screened by STZ induced diabetes in rats model. The result is tabulated in Table no-9 and graphically represented in fig no-27. The result revealed that, the final formulation shows better diabetic control over other prepared formulations.

Conflicts of Interest: The authors declare no Conflict of Interest

Acknowledgement: Authors are thankful to Sun pure extract Pvt Ltd for providing Curcumin and Piperine as gift sample for this research work. The authors are also thankful to Principal and management of Columbia Institute of Pharmacy, Raipur, C.G., Principal, Faculty of Pharmacy, Kalinga University, Raipur and Dean, College of Veterinary Science and Animal Husbandry, Anjora-491001, Durg, C.G., India for providing necessary facilities to complete this research work.

REFERENCES

1. Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, Ganai BA. Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition. World J Diabetes 2015; 6(4): 598-612. https://doi.org/10.4239/wjd.v6.i4.598 PMid:25987957 PMCid:PMC4434080

2. Satapathy T, Mishra G, Panda PK. Animal models for Type-2 diabetes: A review. World Journal of Pharmaceutical Sciences.2016; 4(1):141-147.

3. Dandona L. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990-2016. Lancet Public Health. 2018; 3: 478-89. https://doi.org/10.1016/S2468-2667(18)30138-5 PMid:30219340

4. Andersen DK , Sandberg AA, Duell EJ, Goggin M , Korc M , Petersen GM et.al. Whitcomb, Pancreatitis-diabetes-pancreatic cancer: summary of an NIDDK-NCI Workshop, Pancreas.(2013); 42 (8):1227-1237. https://doi.org/10.1097/MPA.0b013e3182a9ad9d PMid:24152948 PMCid:PMC3878448

5. Sinha D, Satapathy T, Dewangan MK, Kumar A, Roy A. Recent status on carbohydrate metabolizing enzyme inhibitors in regulation of diabetes: a mechanism based review. Journal of applied Pharmaceutical Research. 2015;3(2):1-7.

6. Matveyenko AV, Dry S, Cox Moshtaghian Gurlo TA, Galasso R et.al. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin, Diabetes. (2009); 58 (7):1604-1615. https://doi.org/10.2337/db09-0058 PMid:19403868 PMCid:PMC2699878

7. Manach C, Scalbert A, Morand C. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004;79:727-747. https://doi.org/10.1093/ajcn/79.5.727 PMid:15113710

8. Ammon HP, Wahl MA. Pharmacology of Curcuma longa. Planta Med. 1991; 57:1-7. https://doi.org/10.1055/s-2006-960004 PMid:2062949

9. Rajasekaran SA. Therapeutic potential of Curcumin in gastrointestinal diseases. World J Gastrointest Pathophysiol 2011; 2(1):1-14. https://doi.org/10.4291/wjgp.v2.i1.1 PMid:21607160 PMCid:PMC3097964

10. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: problems and promises. Mol Pharm 2007; 4(6):807-818. https://doi.org/10.1021/mp700113r PMid:17999464

11. Aggarwal BB, Harikumar KB. Potential therapeutic effects of Curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2009; 41:40. https://doi.org/10.1016/j.biocel.2008.06.010 PMid:18662800 PMCid:PMC2637808

12. Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian Pacific Journal of Tropical Biomedicine, 2013; 3(4):253-266. https://doi.org/10.1016/S2221-1691(13)60060-X PMid:23620848

13. Mosallanezhad P, Nazockdast H, Ahmadi Z, Rostami A. Fabrication and characterization of Polycaprolactone/Chitosan nanofibers containing antibacterial agents of Curcumin and ZnO nanoparticles for use as wound dressing. Bioeng.Biotechnol.2022; 10:1-14. https://doi.org/10.3389/fbioe.2022.1027351 PMid:36213068 PMCid:PMC9539460

14. Satapathy T, Panda PK, Goyal AK, Rath G. Evaluation of anti-GERD activity of gastro retentive drug delivery system of itopride hydrochloride. Artificial Cells, Blood Substitutes, and Biotechnology. 2010; 38 (4):200-207. https://doi.org/10.3109/10731191003776751 PMid:20515421

15. Satapathy T and Panda PK. Solid Lipid Nanoparticles: A Novel Carrier in Drug Delivery System.2013; 5(2):56-61.

16. Elavarasan A. EDX and XRD, FT-IR spectra, analysis containing hexavalent chromium metal ion adsorption present in aqueous solution on to phosphoric acid (h 3 po 4) activated Mimusops elengi leaves carbon. Journal of Drug Delivery & Therapeutics. 2018; 8(5-s):132-138. https://doi.org/10.22270/jddt.v8i5-s.1917

17. Togbossi LA, Lawson-Evi P, Atchou NK, Motto EA et.al. In vivo assessment of acute and sub chronic toxicity of hydro ethanolic extract of Ximenia americana L. (Olacaceae) stem bark. Journal of Drug Delivery & Therapeutics. 2022; 12(4):81-86. https://doi.org/10.22270/jddt.v12i4.5567