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RESEARCH ARTICLE

Formulation and Evaluation of Herbal Silver Nanoparticle

Mr. Mirza Nazish Baig[1] and Mr. Ramdas Dolas[2]
[1]Research Scholars, School of Pharmaceutical Science, Sundip University, Nashik (M.S.)
[2]Associate Professor, School of Pharmaceutical Science, Sandip University, Nashik (M.S.)

*Corresponding Author
Mr. Mirza Nazish Baig
(mirzanazishbaig.mnb@gmail.com)

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Abstract: Nanoparticle is the targeted drug delivery system. The research work is done and formulation of the silver nanoparticle was performed. In all four batches was formulated (Three batch of each plant extract and a combination of all three extract). The nanoparticle was prepared by Appling Qbd (Design Expert software). All nanoparticle was evaluated for the UV- Visible spectroscopy, Particle Size, SEM, TEM. Futhermore the nanoparticle was evaluated for In Vitro Antioxidant Activity Assays, DPPH Radical Scavenging Assay, ABTS Radical Scavenging Assay, Total Antioxidant Capacity, Pharmacological Activities, Acute Toxicity Studies, Body Weights and Organ Weights, SGPT (IU/L), SGOT (IU/L), ALP (IU/L), Total Protein, Bilirubin, Creatinine, Histopathological studies of the liver.

Keyword: Nanoparticle, SGOT, SGPT, Histology.

INTRODUCTION

Nanotechnology is associated with nano-meter sized objects. Living organisms are made up of cells. These cell parts, however, are nano sized. Nanotechnology basically deals with design, production and characterization on nano sized particles. Nano sized particles are basically small objects that act as a whole unit in accordance with their transport and properties. Fine particles have the range of 100-2500nm and ultrafine particles have the size of 1- 100nm. They can also be designed to improve the pharmacological and therapeutic effects of the drugs.

Types of nanoparticles: Inorganic nanoparticle: In the field of Modern material science Inorganic nanoparticle has been developed the role based upon their unique physical properties and particularly in biotechnology. Based upon these two factors of inorganic nanoparticles they have certain physical properties that mainly include size dependent optical, magnetic, electronic, and catalytic properties. Bio related application are involved for the preparation of these interesting nanoparticles like iron oxides, gold, silver, silica, quantum dots etc. Novel physical properties mainly related because of their size approaches nanometer scale dimension.

Polymeric nanoparticles: Polymeric nanoparticle it is also a type of nanoparticle. In the recent year polymeric nanoparticle has a tremendous development in the field of research. The dispersion of preformed polymers and the polymerization of monomers are two strong strategies mainly involved for preparation. 10 1000nm it is the range of size involved with solid particles.

Solid lipid nanoparticles: For controlling the drug delivery in 1990 s Solid lipid nanoparticles played a dominant role. There are certain alternate carrier systems to emulsions, liposomes and polymeric nanoparticles as a colloidal Carrier system.

Liposomes: Liposomes are one of the methods based upon the different types of nanoparticles. Structure of liposomes consists of one or more phospholipid bilayers and they are sphere-shaped vesicles to carry compound of interest. Today liposomes have been useful in the field of reagent and tool in various scientific disciplines. Since many features involved in liposome they made their own way in the market. Cosmetic and pharmaceutical industries numerous molecules act as a carrier, and in the field of Food and farming industries liposomes involved in encapsulation to grow delivery system that can entrap unstable compounds.

Nanocrystal A nanocrystal is a type based upon material particle having at least one dimension smaller than 100 nanometres and mainly composed of atoms in either a single or poly-crystalline arrangement. Nanocrystals are aggregates of around hundreds or thousands of molecules that combine in a crystalline form, composed of pure drug with only a thin coating comprised of surfactant or combination of surfactants.

Nanotube: A nanotube is a nanometer scale tube like structure. Nanotubes are members of the fullerene structural family. Their name is derived from their long, hollow structure with the walls formed by one-atom-thick sheets of carbon called graphene. These sheets are rolled at specific and discrete ("chiral") angles and the combination of the rolling angle and radius decides the nanotube properties; for example, whether the individual nanotube shell is a metal or semiconductor. Nanotubes are categorized as single-walled nanotubes (SWNTs) and multi-walled nanotubes.

Dendrimers: Dendrimers arise from two Greek words: Dendron meaning tree and Meros meaning part. Structure of dendrimers has a well-defined size, shape and defined molecular weight and also Dendrimers are hyperbranched, globular, monodisperse, three dimensional nanoscales synthetic Polymers. Molecular chemistry and polymer chemistry both exhibit well-defined characteristics features of Dendrites.

Advantages: Some of the advantages of using nanoparticles as a drug delivery system are as follows; **1.** Ease of manipulation of the particle size and surface characteristics of nanoparticles so as to achieve both passive and active drug targeting after parenteral administration. **2.** The nanoparticle surface can be modified to alter biodistribution of drugs with subsequent clearance of the drug so as to achieve maximum therapeutic efficacy with minimal side effects of the drug. **3.** Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. **4.** Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity. **5.** Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.**6.** Liposomes and polymer based nanoparticulates are generally biodegradable, do not accumulate in the body and so are possibly risk free. **7.** Small sized nanoparticles can penetrate through smaller capillaries, which could allow efficient drug accumulation at the target sites. **8.** Various routes of administration are available including oral, nasal, parenteral, intra-ocular etc.

Limitations: In spite of these advantages nanoparticles do have limitations like, 1. Altered physical properties which lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms due to smaller size and larger surface area. 2. Smaller the particles size greater the surface area and this property makes nanoparticles very reactive in the cellular environment. 3. Small particles size results in limited drug loading and burst release. These practical problems have to be sorted out before nanoparticles can be used clinically or made commercially available.

Green synthesis of nanoparticles [15,16] : Three melting states of nanoparticles have been selected for green or environmental conditions, which is a reduction in efficiency, similar to that of painless. For the synthesis of nanoparticle particles, various synthetic materials have been used where tissue, synthetics and materials are present. As a system, combined strategies are as expensive as the use of hazardous and potentially synthetic chemicals for various hazards in the climate. The utilisation of plants and microbes for biomedical applications protects biosynthesis, which is biocompatible and safe in environmental technologies for the integration of nanoparticles. Growth, green growth, bacteria, plants, and other factors can all be used to make this link. Because of the existence and nature of phytochemicals that function as stimulants and decrease movement, several plant components such as leaves, natural products, roots, and fruits have been utilised to add various nanoparticles[16].

Synthesis of Silver Nanoparticle: Materials: All the glasswares (Borsile) were washed with dilute nitric oxide followed by double distilled water and dried in hot air oven.

Chemical reagents: Ethanol, Silver nitrate ($AgNO_3$), Double distilled water**.**

Instruments Required: Centrifuge, Magnetic stirrer with hot plate, Lyophilizer, UV-Visible spectrophotometer (Lasany), Zetasizer (Malvern Machine, UK), FT-IR (Shimadzu, Japan).

Preparation of 1mM silver nitrate aqueous solution ($AgNO_3$): An accurately weighed 0.017g of silver nitrate was dissolved with 100mL of double distilled water and stored in amber colour bottle until further use.

Synthesis of Silver Nanoparticle using Hydroalcoholic Stem extracts of Tinospora Cordifolia: 5ml of the Hydroalcoholic stem extract of Tinospora Cordifolia was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50ml of 1mM AgNO3 solution was added with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 4-5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the Hydroalcoholic extract of Tinospora Cordifolia generate extremely stable silver nanoparticle.

Synthesis of Silver Nanoparticle using Alcoholic extract of Tephrosia Perpurea: 5ml of the Alcoholic extract of Tephrosia Perpurea was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50ml of 1mM $AgNO_3$ solution was added with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 4-5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the Alcoholic extract of Tephrosia Perpurea generate extremely stable silver nanoparticle.

Synthesis of Silver Nanoparticle using Alcoholic extract of Boerhavia diffusa: 5ml of the Alcoholic Plant extract of Boerhavia diffusa was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50ml of 1mM $AgNO_3$ solution was added with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to

brown after 4-5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the Alcoholic extract of Boerhavia diffusa generate extremely stable silver nanoparticle.

Synthesis of Silver Nanoparticle using Combined extracts of Tinospora Cordifolia, Tephrosia Perpurea and Boerhavia diffusa Silver Nanoparticle: 5ml of each extract was taken in the conical flask and placed on a magnetic stirrer with hot plate. To this 150ml of 1mM $AgNO_3$ solution was added with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 4-5h was observed which indicated the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by the Alcoholic extract of Boerhavia diffusa generate extremely stable silver nanoparticle

Characterization of Tinospora Cordifolia, Tephrosia Perpurea and Boerhavia diffusa Silver Nanoparticle: UV-Visible spectroscopy: The formation and completion of silver nanoparticles was characterized by UV-Visible spectroscopy by using Microprocessor double beam spectrophotometer (Lasany). The bio-reduction of the Ag+ ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function of time of reaction in 300-800nm range operated at a resolution of 1nm. Distilled water was used as a blank.

Dynamic Light Scattering (DLC) Analysis: The particle size and zeta potential characterization and poly dispersibility index of colloidal dispersion of Silver Nanoparticle was investigated using Zetasizer (Malvern Machine, UK). Silver Nanoparticle was diluted in deionized water before analyzed.

Scanning Electron Microscopy: The SEM image showing the high density Ag-NPs synthesized by using the Stem extract of Tinoporia Cordifolia, Entire plant extract of Teprosia Perpuria, Entire plant extract of *Boerhaavia diffusa and Combined* extract further confirmed the development of silver nanostructures. Obtained nanoparticle showed that Ag-NPs are spherical shaped and monodispersed and well distributed with aggregation.

Transmission Electron Microscope (TEM): To obtain Sharpe images from a TEM (Hitachi-H-7500) 120 kV equipped with CCD camera, a sample of synthesized SNPs was spotted onto a carbon-coated copper grid and scanned via 1,50,000x to 3,00,000x magnification.

In Vitro Antioxidant Activity Assays: The antioxidant potential of the prepared nanoparticle formulations and the polyherbal extract was evaluated using three established in vitro assays.

DPPH Radical Scavenging Assay: The DPPH radical scavenging activity was determined spectrophotometrically according to the method of "Blois (1958) with slight modifications. Briefly, (1.0, 1.5, and 2.0 mg/mL) were mixed with a 0.1 mM DPPH solution in ethanol. The mixture was incubated in the dark for 30 minutes at room temperature, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (model, manufacturer). Ascorbic acid served as the positive control. The percentage of DPPH scavenging was calculated using the formula: **% Scavenging = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100]. ABTS Radical Scavenging Assay:** The ABTS radical scavenging activity was measured using the method "The ABTS radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The ABTS solution was then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Sample aliquots (1.0, 1.5, and 2.0 mg/mL) were added to the diluted ABTS solution, and the absorbance was recorded at 734 nm after 6 minutes. Ascorbic acid was used as the positive control. The percentage of ABTS scavenging was calculated using the same formula as for DPPH. Total Antioxidant Capacity (TAC): The total antioxidant capacity was determined by the phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) by the sampleAn aliquot of the sample (1.0, 1.5, and 2.0 mg/mL) was combined with the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes. After cooling, the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as mg of ascorbic acid equivalent per gram of sample (mg AAE/g) using an ascorbic acid standard curve**.**

Animal Studies: Animals: We utilized albino rats weighing 150-250 g that came from reputable suppliers. The animals were housed in a typical setting, given a typical pellet diet, and allowed unfettered access to water and food after receiving clearance from the Institutional Animal Ethics committee of **Loknete Shri Dadapatil Pharate College of Pharmacy. SWGP/LSDP/PHARMA/IAEC/2024-2025/18.Chemicals and standard drug:** Silymarin **(Sydler Remedies Pvt Ltd)** CCL_4 , normal saline solution was procured and used.

Determination of Acute toxicity (LD50). Preparation of dose: The nanoparticle formulations of herbal extracts were made at a dosage of 2000 mg/kg body weight and given to the animals at a rate of 1ml/100 g body weight. In order to establish the safe dosage, formulations were tested for acute toxicity using albino male rats in accordance with the acute toxic classic technique (OECD guideline 423).

Animals: Male albino rats (150 -250 g) will be used in the study and kept fasting overnight with free access to water. During the experiments, the animals were divided into seven groups of six animals in each group.

Table: Experimental Design

Group	Treatment	No of animals
Group 1 Normal control	normal saline	6
Group 2 toxic control	CCl4 (3mg/kg)	6
Group 3 Standard control	Silymarin (25mg/kg)+CCl4(3mg/kg)	6
Group 4 Tinosproa Cordifolia Nanoparticle	PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	6
Group 5 Tephrosia Perpurea Nanoparticle	PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	6
Group 6 Boerhavia Diffusa Nanoparticle	PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	6
Group 7 Polyherbal extract Nanoparticle	PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	6

PHF-plant herbal formulation

Collecting Blood for Biochemical Analysis: For biochemical analysis that followed, blood samples were taken by retro-orbital puncture. After blood samples were taken, they were left to coagulate for half an hour at room temperature. A series of biochemical parameters were estimated from the serum samples that had been centrifuged at 3000 rpm for 15 minutes to separate the components.

PREPARATION OF LIVER SAMPLES Blood samples were obtained via retro-orbital puncture for subsequent biochemical analysis. Following collection, the blood was permitted to clot at room temperature for 30 minutes. Serum was then separated by centrifugation at 3000 revolutions per minute for 15 minutes, and these samples were subsequently used for the estimation of various biochemical parameters.

EVALUATION OF CLINICAL PARAMETERS

- Liver enzyme
- Total Protein
- Total Bilirubin

Statistical analysis: Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, $p<0.001$; when compared to toxic control, $p>0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

RESULT:

Formulation Batch containing three Extracts Using Experimental Design: Experimental Design of Batch Containg 3 Extracts

Std	ID	Block	Group	Run	Build Type	Space Type	Factor 1: A:Conc. of AgNO3 (mM)	Factor 2: B:Extract Volume (ml)	Factor 3: C:Temp. (^{0}C)	Response 1:Particle Size (nm)	Response 2: Zeta Potential (mV)
8	8	Block 1	1	1	NA	Factorial	2	60	65	111.35	-23.68
1	1	Block 1	1	2	NA	Factorial	0.5	30	45	80.64	-28.35
6	6	Block 1	1	3	NA	Factorial	2	30	65	94.15	-18.57
7	7	Block 1	1	4	NA	Factorial	0.5	60	65	135.31	-10.22

2	2	Block 1	1	5	NA	Factorial	2	30	45	85.44	-16.51
3	3	Block 1	1	6	NA	Factorial	0.5	60	45	120.75	-11.42
5	5	Block 1	1	7	NA	Factorial	0.5	30	65	109.58	-13.35
4	4	Block 1	1	8	NA	Factorial	2	60	45	114.96	-8.83

Table no. 4: Formulation Batch containing three Extracts Using Experimental Design:

Sr.No	Ingredients	I	II	III	IV	V	VI	VII	VIII
1	**Conc. of AgNO3 (mM)**	2	2	2	2	2	2	2	2
2	**Extract Volume (ml)**	60	60	60	60	60	60	60	60
3	**Temperature (^{0}C)**	65	65	65	65	65	65	65	65

Particle size

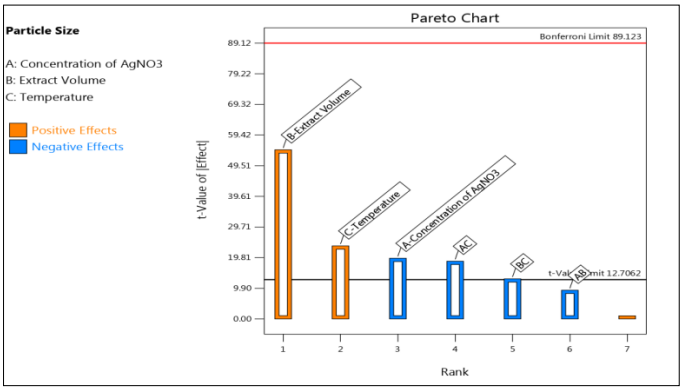
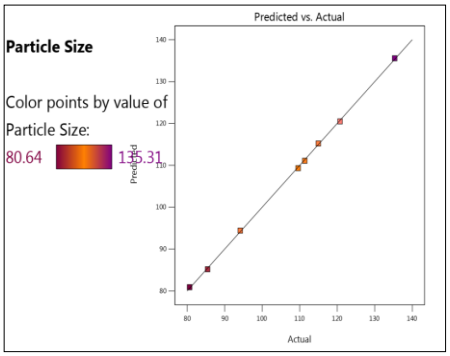


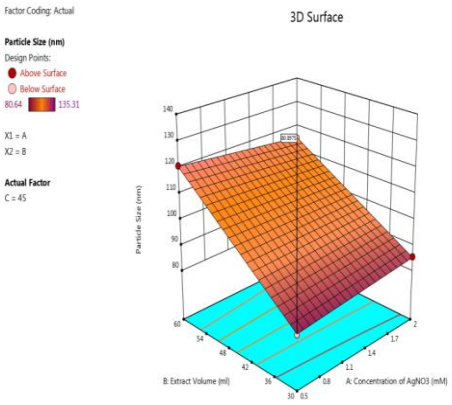
Table no. 5: ANOVA for selected factorial model

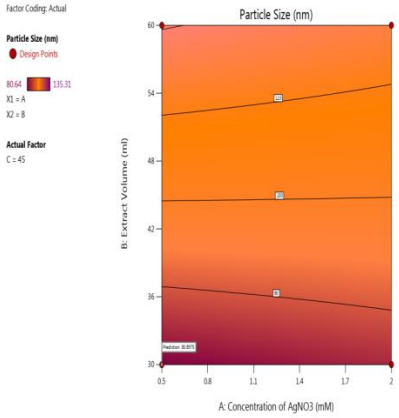
Response 1: Particle Size

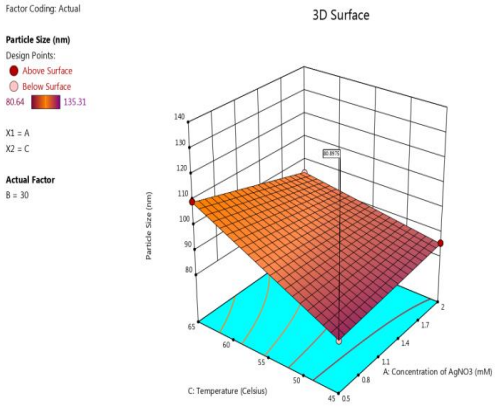
Source Model	Sum of Squares	df	Mean Square	F-value	p-value	
	2401.91	6	400.32	754.68	0.0279	**significant**
A-Conc. of $AgNO_3$	203.82	1	203.82	384.24	0.0324	
B-Extract Volume	1583.72	1	1583.72	2985.61	0.0116	
C-Temp.	295.24	1	295.24	556.59	0.0270	
AB	45.70	1	45.70	86.15	0.0683	
AC	184.32	1	184.32	347.48	0.0341	
BC	89.11	1	89.11	167.99	0.0490	
Residual	0.5304	1	0.5304			
Cor Total	2402.44	7				

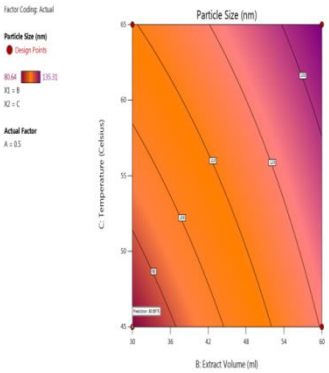
Factor coding is **Coded**. Sum of squares is **Type III - Partial**

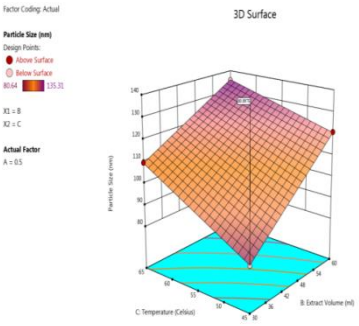


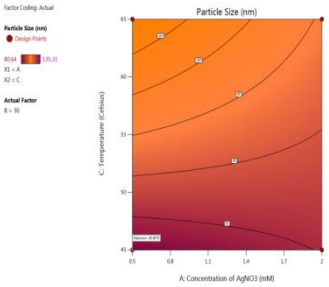












Zeta Potential

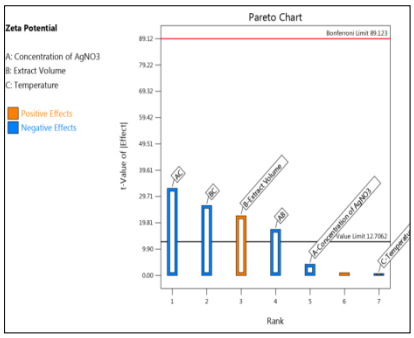
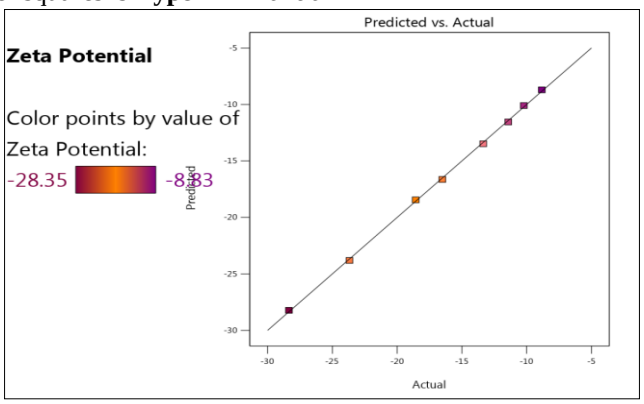


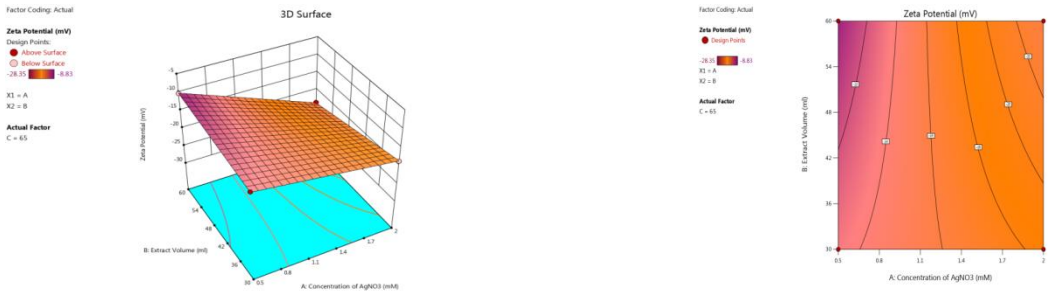
Table no. 06: ANOVA for selected factorial model :

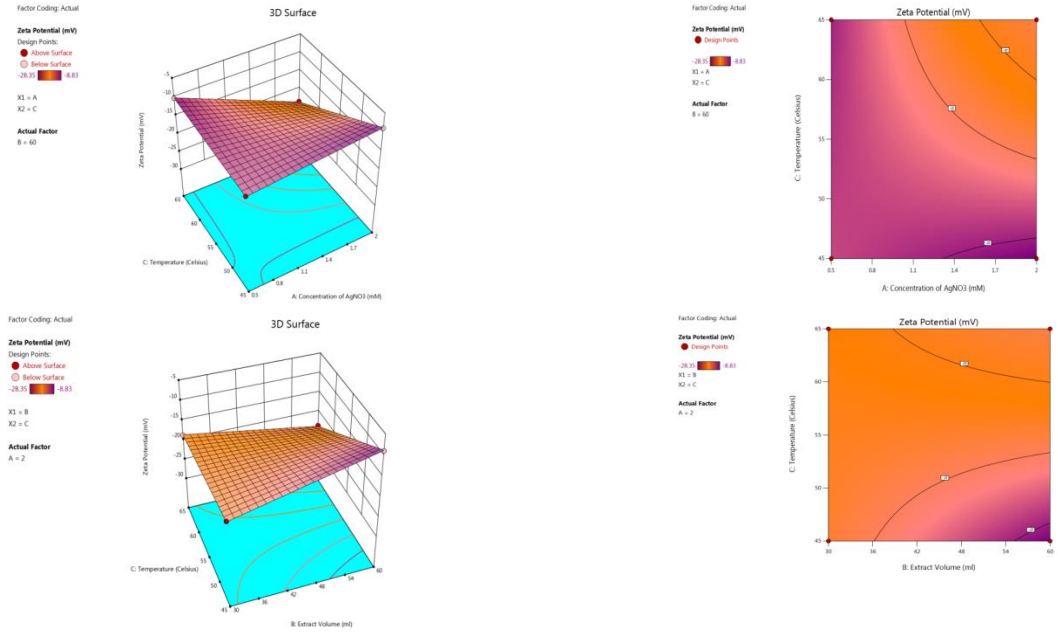
Response 2: Zeta Potential

Source Model	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
	329.99	6	55.00	431.31	0.0368	**significant**
A-Conc. of $AgNO_3$	2.26	1	2.26	17.71	0.1485	
B-Extract Volume	64.01	1	64.01	502.03	0.0284	
C-Temp.	0.0630	1	0.0630	0.4942	0.6099	
AB	38.24	1	38.24	299.87	0.0367	
AC	137.03	1	137.03	1074.67	0.0194	
BC	88.38	1	88.38	693.10	0.0242	
Residual	0.1275	1	0.1275			
Cor Total	330.11	7				

Factor coding is **Coded**. Sum of squares is **Type III - Partial**







71 Solutions found. Each solution is then tested in the experiment, and the corresponding response variable(s) are measured or observed. By systematically varying the factors and observing the responses, researchers can analyze how changes in factors affect the outcome. The perfect solution can be linked to desirability functions. Desirability functions are used to assess the desirability of a set of responses based on specified criteria or goals. They allow researchers to combine multiple responses into a single desirability score, which represents how well the experimental conditions meet the desired objectives. Desirability scores are calculated for each response using desirability functions. These functions transform individual responses into desirability scores ranging from 0 to 1, where 1 represents the ideal value and 0 represents the least desirable value.The solution with the highest overall desirability score is chosen as the optimized batch. This solution represents the best combination of factor levels that maximizes the desirability of the experimental outcomes, taking into account the specified objectives and constraints. From all 71 solutions, solution 1 has desirability score is around 0.955 which is highest and closer to the 1 so the solution 1 is considered as the optimized batch from all solutions. So, this batch is further formulated, analyzed for the all necessary parameters and compared to the predicted batch given by the software.

Table no. 07: Optimized batch given by the software:

Concentration of $AgNO_3$ (mM)	**Extract Volume (ml)**	**Temperature (°C)**	**Particle Size (nm)**	**Zeta Potential (mV)**
0.500	30.000	45.000	80.897	-28.224

When we formulate this batch the Particle size and Zeta potential of silver nanoparticles will be predicted 80.897 nm and -28.224 mV respectively.

Characterization of Tinospora Cordifolia, Tephrosia Perpurea and Boerhavia diffusa Silver Nanoparticle: UV-Visible spectroscopy:

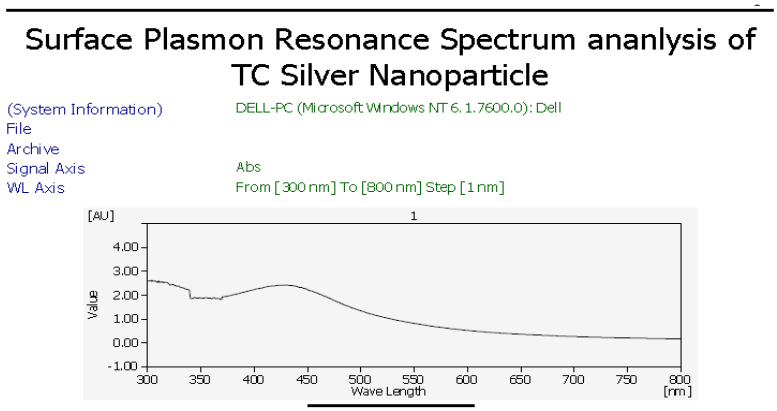


Figure1: SPR Spectrum analysis of TC Silver Nanoparticle by using UV-Vis Spectroscopy

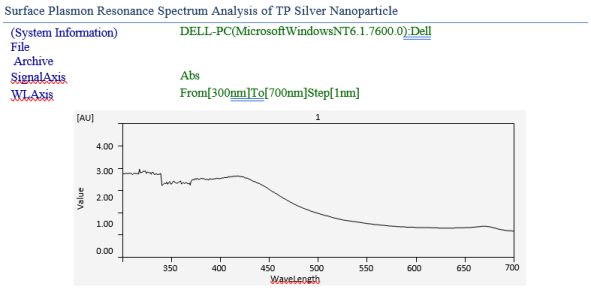


Figure2: SPR Spectrum analysis of TP Silver Nanoparticle by using UV-Vis Spectroscopy

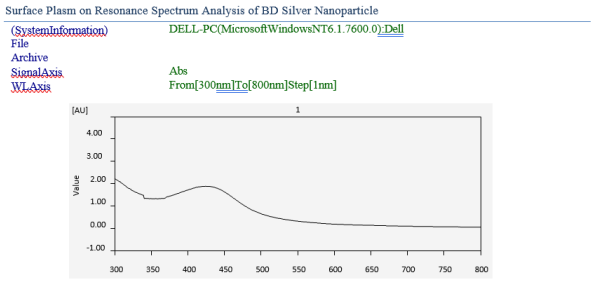


Figure3: SPR Spectrum analysis of BD Silver Nanoparticle by using UV-Vis Spectroscopy

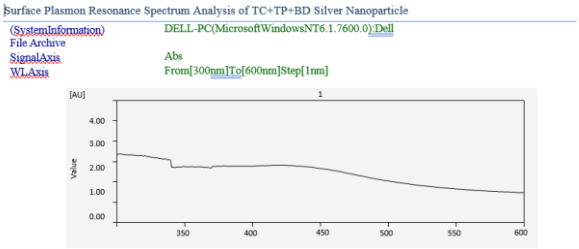
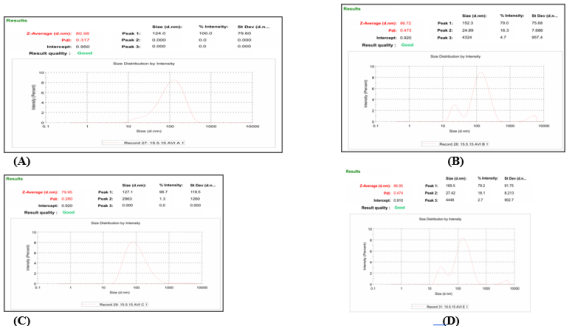


Figure4: SPR Spectrum analysis of Combined Extract (TC+TP+BD) Silver Nanoparticle by using UV-Vis Spectroscopy

The UV-Visible Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The reduction of the pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 4-5 hours (complete colour change) following the dilution of a small aliquot of the sample in distilled water. The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Vis Spectra of the colloidal solution which exhibited a strong absorption at 430nm shown in Figure. A typical peak was obtained due to the presence of surface Plasmon resonance silver nanoparticle.

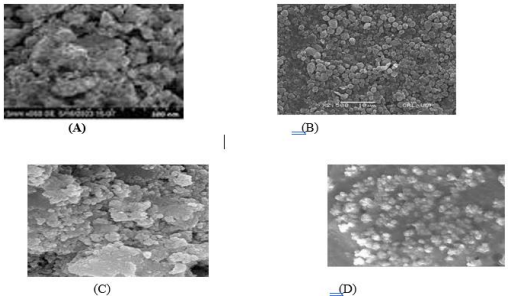
Particle Size Determination:



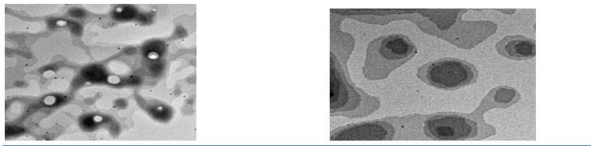
Particle Size Determination: (A) Tinospora Cordifolia (B) Tephrosia Perpura, (C) Borhevia Diffisa, (D) Combination

The Particle Size of the Tinospora Cordifolia, Tephrosia Perpura, Borhavia Diffusia and Combined extract was determined and the observation was found to be Good.

Scanning Electron Microscope:



(A): SEM Image of Tinopora Cordifolia, (B): SEM Image of Tephrosia Perpuria, (C): SEM Image of Borhavia Diffusia, (D): SEM Image of Combination Extracts.



TEM of Final Combination Formulation

In- vitro antioxidant activity: DPPH Radical Scavenging (%): The in-vitro antioxidant activity of various nanoparticle formulations and a polyherbal extract was evaluated using the DPPH radical scavenging assay, with ascorbic acid serving as a standard reference. The results demonstrate a clear concentration-dependent increase in DPPH radical scavenging activity for all tested samples. Among the individual nanoparticle formulations, Tinospora Cordifolia nanoparticles exhibited the highest antioxidant potential, achieving 68 ± 2.50% scavenging at 2.0 mg/mL. This was followed by Tephrosia Purpurea nanoparticles (57 ± 3.00% at 2.0 mg/mL) and Boerhavia Diffusa nanoparticles (41 ± 3.50% at 2.0 mg/mL). The polyherbal extract nanoparticle showed the lowest scavenging activity among all tested formulations, reaching only 38 ± 2.30% at the highest concentration. Notably, Tinospora Cordifolia nanoparticles at 2.0 mg/mL displayed comparable antioxidant activity to ascorbic acid at 1.0 mg/mL (42 ± 2.93%), and were approaching the activity of ascorbic acid at 1.5 mg/mL (63 ± 1.76%), indicating a strong antioxidant capacity. Ascorbic acid, as expected, demonstrated the most potent radical scavenging activity, achieving 76 ± 1.86% at 2.0 mg/mL.

Table no.08: DPPH Radical Scavenging (%)

Sample	Concentration (mg/mL)	DPPH Radical Scavenging (%)
Tinosproa Cordifolia Nanoparticle	1	37 ± 1.50
	1.5	53 ± 2.00
	2.0	68 ± 2.50
Tephrosia Perpurea Nanoparticle	1	33 ± 2.00
	1.5	42 ± 2.50
	2.0	57 ± 3.00
Boerhavia Diffusa Nanoparticle	1	22 ± 2.50
	1.5	34 ± 3.00
	2.0	41 ± 3.50
Polyherbal extract Nanoparticle	1	18 ± 1.20
	1.5	28 ± 1.80
	2.0	38 ± 2.30
Ascorbic acid	1	42 ± 2.93
	1.5	63 ± 1.76
	2.0	76 ± 1.86

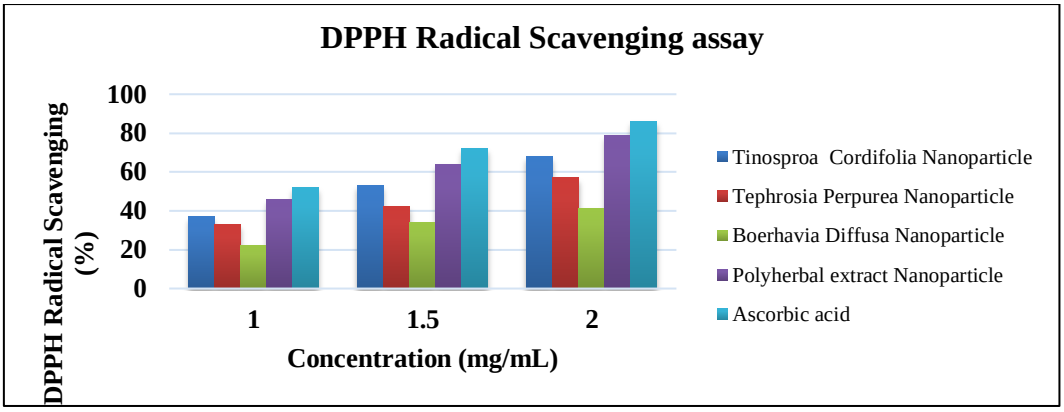


Figure no.09: Bar Chat of DPPH Radical Scavenging assay

ABTS Radical Scavenging (%): The ABTS radical scavenging activity of the various nanoparticle formulations and the polyherbal extract was also assessed, with ascorbic acid serving as a positive control. Similar to the DPPH assay, all samples demonstrated a concentration-dependent increase in ABTS radical scavenging activity. Interestingly, the trend observed in the ABTS assay differs from that of the DPPH assay concerning the top-performing samples.In the ABTS assay, the polyherbal extract nanoparticle exhibited the highest scavenging activity among all the tested nanoparticle formulations, reaching 68 ± 2.80% at 2.0 mg/mL. This is a notable improvement compared to its performance in the DPPH assay. Following the polyherbal extract, Tinospora Cordifolia nanoparticles showed strong activity (61 ± 3.00% at 2.0 mg/mL), followed closely by Tephrosia Purpurea nanoparticles (55 ± 3.50% at 2.0 mg/mL) and Boerhavia Diffusa Nanoparticle (53 ± 4.00% at 2.0 mg/mL). Ascorbic acid consistently displayed superior antioxidant activity, achieving 89 ± 2.83% ABTS radical scavenging at 2.0 mg/mL. The polyherbal extract nanoparticle at 2.0 mg/mL showed a comparable scavenging capacity to ascorbic acid at 1.0 mg/mL (56 ± 3.14%), indicating its significant antioxidant potential in this assay

Table no.09: ABTS Radical Scavenging (%)

Sample	Concentration (mg/mL)	ABTS Radical Scavenging (%)
Tinosproa CordifoliaNanoparticle	1	42 ± 2.00
	1.5	53 ± 2.50
	2.0	61 ± 3.00
*Tephrosia Perpurea*Nanoparticle	1	35 ± 2.50
	1.5	45 ± 3.00
	2.0	55 ± 3.50
*Boerhavia Diffusa*Nanoparticle	1	34 ± 3.00
	1.5	46 ± 3.50
	2.0	53 ± 4.00
Polyherbal extract Nanoparticle	1	48 ± 1.80
	1.5	59 ± 2.30
	2.0	68 ± 2.80
Ascorbic acid	1	56 ± 3.14
	1.5	76 ± 1.02
	2.0	89 ± 2.83

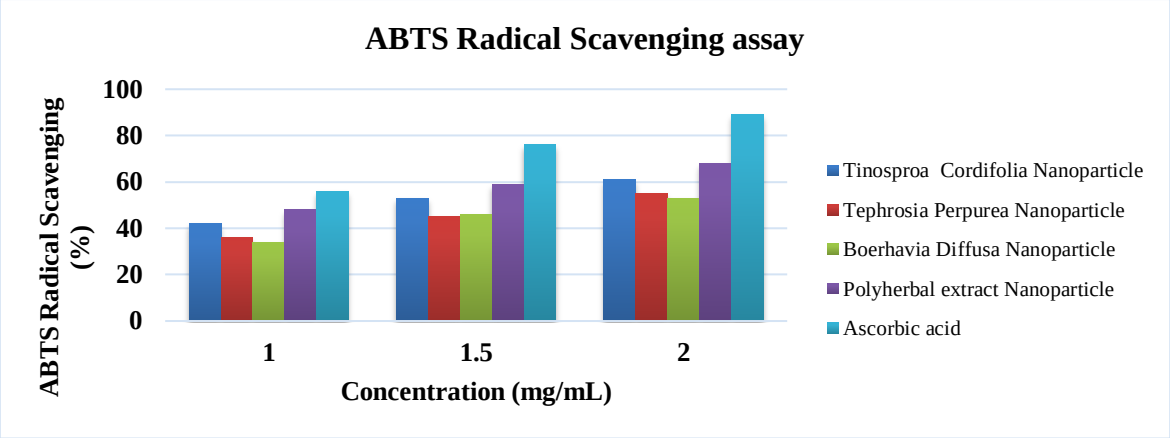


Figure no. 10: Bar Chart of ABTS Radical Scavenging assay

Total Antioxidant Capacity The Total Antioxidant Capacity (TAC) of the various nanoparticle formulations and the polyherbal extract was determined, expressed as milligrams of Ascorbic Acid Equivalent per gram of sample (mg AAE/g). Consistent with the radical scavenging assays, all samples demonstrated a concentration-dependent increase in their total antioxidant capacity. Among the individual herbal nanoparticle formulations, both Tinospora Cordifolia nanoparticles and Boerhavia Diffusa nanoparticles exhibited similar and relatively high total antioxidant capacities, reaching 28 ± 1.50 mg AAE/g and 28 ± 2.00 mg AAE/g respectively at 2.0 mg/mL. Tephrosia Purpurea nanoparticles showed a slightly lower TAC, with 25 ± 2.00 mg AAE/g at the highest concentration. Notably, the Polyherbal extract nanoparticle consistently demonstrated the highest total antioxidant capacity among all the tested nanoparticle formulations, achieving 31 ± 1.30 mg AAE/g at 2.0 mg/mL. This further supports the findings from the ABTS assay, where the polyherbal extract also showed superior activity. This suggests that the combination of herbs in the polyherbal extract may lead to synergistic or additive effects, resulting in a higher overall antioxidant potential as measured by the TAC assay. Ascorbic acid, as the standard, displayed the highest total antioxidant capacity across all concentrations, reaching 54 ± 1.06 mg AAE/g at 2.0 mg/mL. The polyherbal extract nanoparticle's TAC at 2.0 mg/mL (31 ± 1.30 mg AAE/g) was comparable to ascorbic acid at 1.0 mg/mL (32 ± 1.18 mg AAE/g), highlighting its significant antioxidant strength.

Table no. 10: Total Antioxidant Capacity (mg AAE/g)

Sample	Concentration (mg/mL)	Total Antioxidant Capacity (mg AAE/g)
Tinosproa Cordifolia Nanoparticle	1	21 ± 1.00
	1.5	25 ± 1.20
	2.0	28 ± 1.50
Tephrosia Perpurea Nanoparticle	1	18 ± 1.20
	1.5	22 ± 1.50
	2.0	25 ± 2.00
Boerhavia Diffusa Nanoparticle	1	20 ± 1.50
	1.5	24 ± 1.80
	2.0	28 ± 2.00
Polyherbal extract Nanoparticle	1	25± 0.90
	1.5	29 ± 1.10
	2.0	31 ± 1.30
Ascorbic acid	1	32 ± 1.18
	1.5	42 ± 2.07
	2.0	54± 1.06

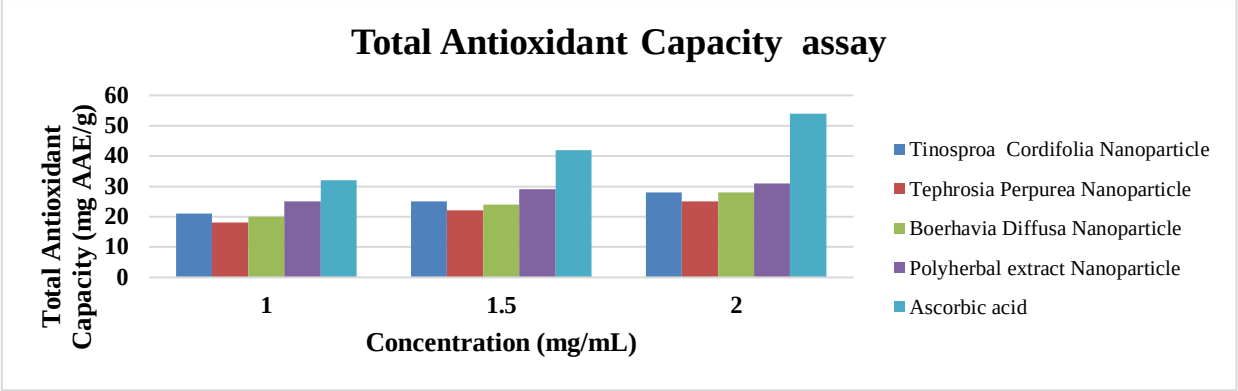


Figure no. 10: Bar Chart of Total Antioxidant Capacity assay

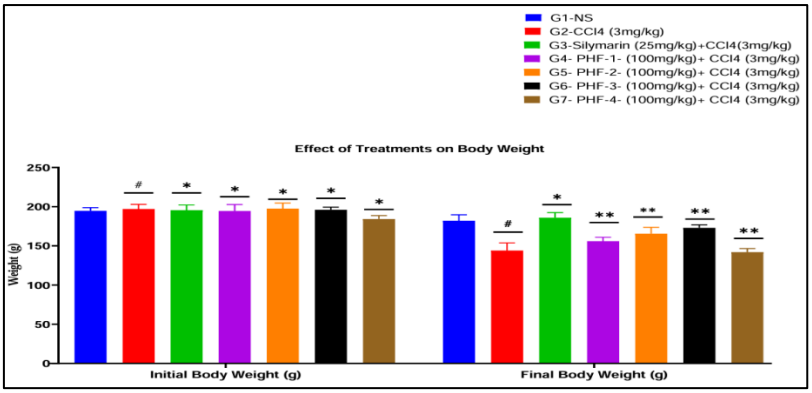
PHARMACOLOGICAL ACTIVITIES: Acute Toxicity Studies: Acute toxicity studies were performed according to the OECD Guideline no. 423. Acute toxicity studies were conducted and no mortality was observedat the dose of 2000mg/kg. Hence 1/ 1/20th of the dose of 2000mg/kg i.e; 100mg/kg has been fixed asED50 for the evaluation of nanoparticle formulation.

Evaluation of Nanoparticle formulations against CCL4 induced Hepatotoxicity: Body Weights and Organ Weights: Effect of Treatments on Body Weight and Liver Weight: The effects of various nanoparticle formulations (PHF-1, PHF-2, PHF-3, PHF-4) on body weight and liver weight were evaluated in a CCl4-induced hepatotoxicity model, with normal saline and silymarin serving as controls. The initial body weights across all groups were comparable, indicating a uniform starting point for the experiment. In the CCl4-intoxicated group (G2), a significant reduction in final body weight (144 ± 9.66 g) was observed compared to the normal control (G1: 182 ± 7.76 g), along with a drastic decrease in liver weight (3.96 ± 3.16 g) due to CCl4-induced liver damage and associated necrosis or atrophy. Treatment with silymarin (G3), a known hepatoprotective agent, effectively prevented these changes, maintaining final body weight (186 ± 6.45 g) and liver weight (6.37 ± 2.30 g) close to normal levels. Similarly, PHF-3 (G6) demonstrated a remarkable protective effect, resulting in a final body weight (173 ± 3.81 g) and liver weight (6.40 ± 1.45 g) that were very close to those of the silymarin-treated and normal control groups. PHF-2 (G5) also showed significant improvement in final body weight (165.56 ± 8.08 g) and liver weight (5.76 ± 2.60 g) compared to the CCl4 group. PHF-1 (G4) provided a moderate protective effect, with a final body weight of 156 ± 4.95 g and liver weight of 5.50 ± 1.34 g. In contrast, PHF-4 (G7) did not show a substantial protective effect on body weight (142 ± 4.60 g) and liver weight (3.70 ± 1.24 g), which remained comparable to the CCl4-intoxicated group.

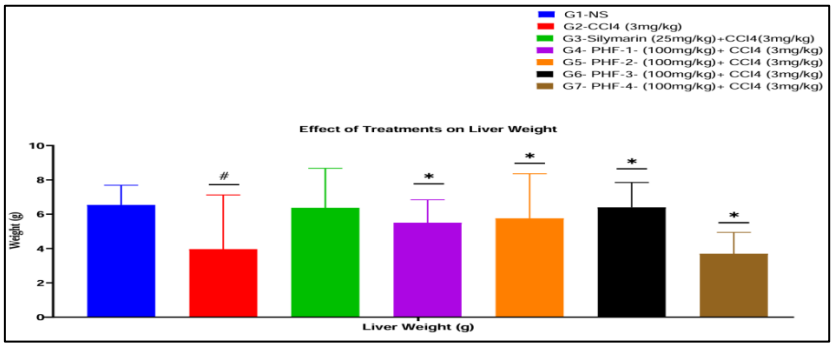
Table no. 11: Effect of Treatments on Body Weight and Liver Weight

Group and Treatment	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (g)
G1- normal saline	194.73 ± 4.94	182 ± 7.76	6.54 ± 1.15
G2- CCl4 (3mg/kg)	197 ± 5.9#	144 ± 9.66#	3.96 ± 3.16#
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	195.5 ± 6.65*	186 ± 6.45*	6.37 ± 2.30
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	194.5 ± 8.33*	156 ± 4.95**	5.50 ± 1.34*
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	197.5 ± 7.09*	165.56 ± 8.08**	5.76 ± 2.60*
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	196 ± 3.36*	173 ± 3.81**	6.40 ± 1.45*
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	184.29 ± 4.33*	142 ± 4.60**	3.70 ± 1.24*

Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, $p<0.001$; when compared to toxic control, $p>0.05$; *$p<0.05$; **$p<0.01$; ***$p<0.001$.



Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.



Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

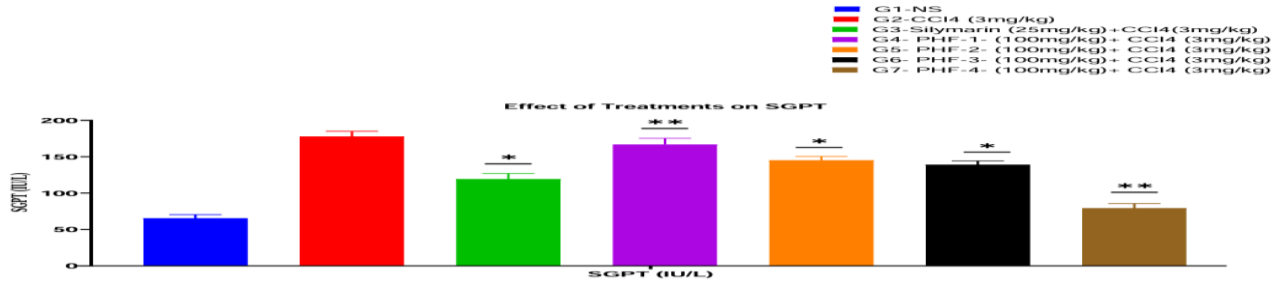
Figure no. 11: Effect of Treatments on Body Weight and Liver Weight

Effect of Treatments on Liver Function Biomarkers: SGPT (IU/L): The impact of the various nanoparticle formulations on liver function was further assessed by measuring serum glutamic pyruvic transaminase (SGPT) levels, a key biomarker for hepatocellular injury. In the normal control group (G1), the SGPT level was 65.5 ± 4.95 IU/L, indicating healthy liver function. As anticipated, CCl4 administration (G2) resulted in a significant elevation of SGPT to 178 ± 6.97 IU/L, confirming the induction of liver damage.Treatment with silymarin (G3), the standard hepatoprotective drug, significantly reduced the CCl4-induced SGPT elevation to 119.3 ± 7.68 IU/L, demonstrating its protective effect. Among the nanoparticle formulations, PHF-4 (G7) exhibited the most remarkable protective effect, reducing SGPT levels to 79.48 ± 6.30 IU/L, which is very close to the normal control group and even surpasses the protective effect of silymarin. This is a significant finding given its less favorable performance in the body and liver weight parameters. PHF-3 (G6) also demonstrated a substantial reduction in SGPT, bringing the level down to 139.2 ± 5.07 IU/L, while PHF-2 (G5) showed a moderate but significant reduction to 145.3 ± 5.07 IU/L. PHF-1 (G4) had the least pronounced effect among the treated groups, with SGPT levels at 167 ± 8.30 IU/L, still significantly higher than the normal and silymarin groups, but showing a slight improvement compared to the CCl4 control.

Table no. 12: Effect of Treatments on Liver Function (SGPT)

Group and Treatment	SGPT (IU/L)
G1- normal saline	65.5 ± 4.95
G2- CCl4 (3mg/kg)	178 ± 6.97
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	119.3 ± 7.68*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	167 ± 8.30**
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	145.3± 5.07*
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	139.2 ± 5.07*
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	79.48 ± 6.30**

Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.



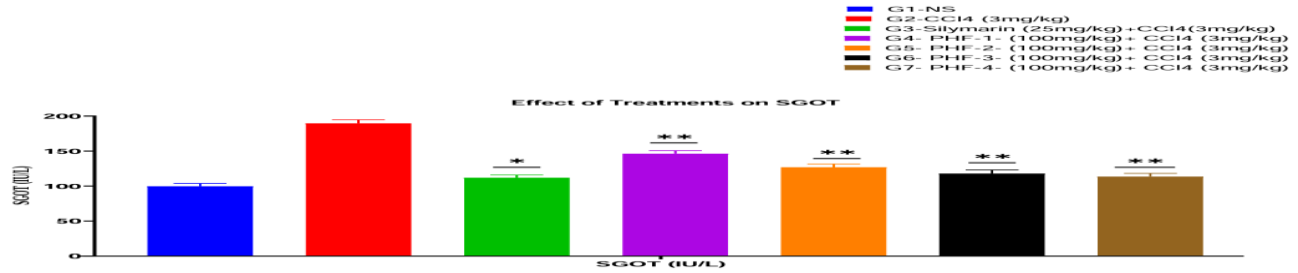
Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

Figure no. 11: Effect of Treatments on Liver Function (SGPT)

SGOT (IU/L): The evaluation of liver function biomarkers continued with the assessment of serum glutamic oxaloacetic transaminase (SGOT) levels. The normal control group (G1) exhibited an SGOT level of 99.78 ± 2.33 IU/L. As expected, CCl4 administration in group G2 led to a significant elevation of SGOT to 189.7 ± 3.18 IU/L, confirming severe hepatocellular damage.Silymarin treatment (G3) effectively mitigated this increase, reducing SGOT levels to 111.9 ± 1.42 IU/L, demonstrating its strong hepatoprotective efficacy. Among the nanoparticle formulations, PHF-3 (G6) and PHF-4 (G7) showed the most significant protective effects, bringing down SGOT levels to 117.9 ± 3.46 IU/L and 113.69 ± 2.63 IU/L, respectively. These values are remarkably close to the silymarin-treated group and represent substantial improvements over the CCl4-intoxicated control. PHF-2 (G5) also demonstrated a notable reduction in SGOT to 126.9 ± 2.36 IU/L. PHF-1 (G4) provided a more modest, but still significant, reduction in SGOT to 146.36 ± 4.23 IU/L compared to the CCl4 control. The consistent and strong performance of PHF-3 and PHF-4 in normalizing both SGPT and SGOT levels strongly indicates their potent ability to preserve hepatocyte membrane integrity and reduce the leakage of intracellular enzymes, thus effectively ameliorating CCl4-induced liver injury.

Table no. 12: Effect of Treatments on Liver Function (SGOT)

Group and Treatment	SGOT (IU/L)
G1- normal saline	99.78 ± 2.33
G2- CCl4 (3mg/kg)	189.7 ± 3.18
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	111.9 ± 1.42*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	146.36 ± 4.23**
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	126.9 ± 2.36**
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	117.9 ±3.46**
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	113.69 ± 2.63**



Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

Figure no. 12: Effect of Treatments on Liver Function (SGOT)

ALP (IU/L): The assessment of liver function extended to Alkaline Phosphatase (ALP) levels, another crucial indicator of hepatobiliary dysfunction. The normal control group (G1) showed an ALP level of 176.73 ± 2.65 IU/L. As expected, CCl4 administration in group G2 significantly elevated ALP to 340.61 ± 5.69 IU/L, indicating severe liver damage and cholestasis. Silymarin treatment (G3) effectively mitigated this increase, reducing ALP levels to 236.51 ± 3.22 IU/L, demonstrating its significant hepatoprotective effect. Among the nanoparticle formulations, PHF-4 (G7) demonstrated an exceptional protective effect, reducing ALP levels to 161.11 ± 2.20 IU/L. This value is not only significantly lower than the CCl4-intoxicated group and the silymarin-treated group, but also falls below the normal control level, suggesting a remarkable ameliorative or even restorative effect on hepatobiliary function. PHF-3 (G6) also showed a very strong protective effect, bringing ALP levels down to 249.51 ± 2.20 IU/L, which is comparable to the silymarin-treated group. PHF-2 (G5) demonstrated a moderate reduction in ALP to 291.52 ± 5.52 IU/L, while PHF-1 (G4) provided the least

significant reduction among the treated groups, with ALP levels at 301.12 ± 4.15 IU/L, though still showing an improvement over the CCl4 control.

Table no. 13: Effect of Treatments on Liver Function (ALP)

Group and Treatment	ALP (IU/L)
G1- normal saline	176.73 ± 2.65
G2- CCl4 (3mg/kg)	340.61 ± 5.69
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	236.51 ± 3.22*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	301.12 ± 4.15*
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	291.52 ± 5.52**
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	249.51 ± 2.20**
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	161.11 ± 2.20*

Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

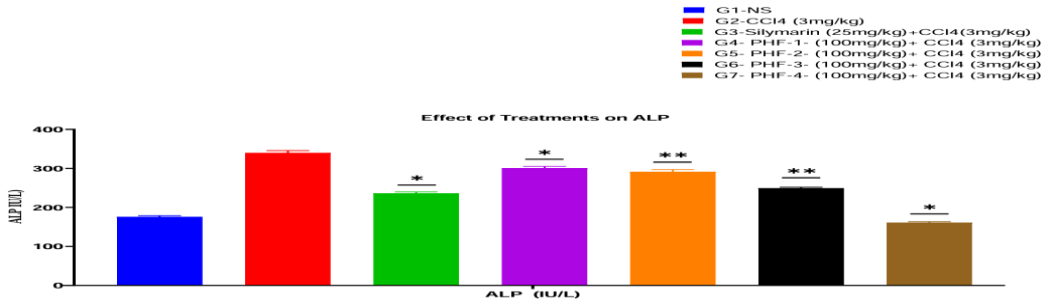


Figure no. 13: Effect of Treatments on Liver Function (ALP)

Total Protein (g/dl): The impact of CCl4-induced hepatotoxicity and the protective effects of the nanoparticle formulations were further assessed by measuring total serum protein levels, an indicator of liver synthetic function. In the normal control group (G1), the total protein level was 8.846 ± 0.008 g/dL. As expected, CCl4 administration in group G2 significantly reduced total protein to 5.875 ± 0.031 g/dL, reflecting impaired protein synthesis due to liver damage. Silymarin treatment (G3) effectively ameliorated this reduction, restoring total protein levels to 7.95 ± 0.02 g/dL, demonstrating its ability to support hepatic protein synthesis. AThis outstanding result suggests a potent restorative effect on liver synthetic function. PHF-2 (G5) also showed a very strong protective effect, significantly increasing total protein to 7.73 ± 0.02 g/dL, closely mirroring the silymarin group. PHF-4 (G7) provided a moderate but significant improvement, raising total protein to 6.83 ± 0.02 g/dL. In contrast, PHF-1 (G4) showed very little protective effect on total protein levels (5.86 ± 0.023 g/dL), remaining largely comparable to the CCl4-intoxicated group.

Table no. 14: Effect of Treatments on Liver Synthetic Function (Total Protein)

Group and Treatment	Total Protein (g/dl)
G1- normal saline	8.846 ± 0.008
G2- CCl4 (3mg/kg)	5.875 ± 0.031#
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	7.95 ± 0.02*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	5.86 ± 0.023*
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	7.73 ± 0.02*
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	8.92 ± 0.02*
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	6.83 ± 0.02*

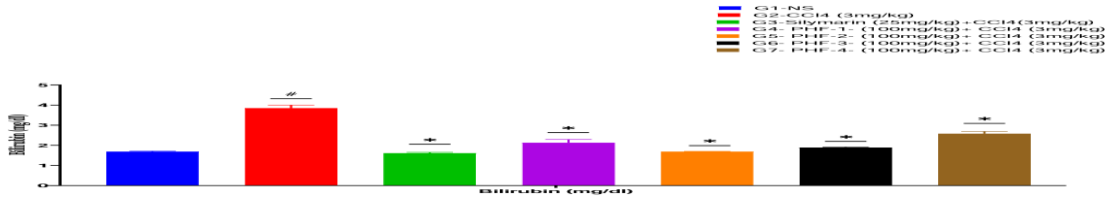
Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

Bilirubin (mg/dl): The effect of CCl4-induced hepatotoxicity and the therapeutic interventions on bilirubin levels, a key indicator of liver's detoxification and excretory function, were assessed. The normal control group (G1) exhibited a bilirubin level of 1.696 ± 0.02 mg/dL. As expected, CCl4 administration in group G2 led to a significant increase in bilirubin to 3.85 ± 0.155 mg/dL, indicating impaired bile excretion and liver dysfunction.Silymarin treatment (G3) demonstrated a strong protective effect, effectively reducing bilirubin levels to 1.61 ± 0.004 mg/dL, which is comparable to or even slightly lower than the normal control group. Among the nanoparticle formulations, PHF-2 (G5) showed the most potent effect in reducing bilirubin, bringing the level down to 1.69 ± 0.01 mg/dL, which is remarkably close to the normal control group and on par with silymarin. PHF-3 (G6) also exhibited a significant reduction in bilirubin to 1.89 ± 0.02 mg/dL, indicating good recovery of excretory function. PHF-1 (G4) provided a moderate but significant improvement, lowering bilirubin to 2.13 ± 0.16 mg/dL. PHF-4 (G7), while showing a significant reduction compared to the CCl4 group, had a less pronounced effect on bilirubin (2.58 ± 0.11 mg/dL) compared to PHF-2 and PHF-3.

Table no. 15: Effect of Treatments on Bilirubin

Group and Treatment	Bilirubin (mg/dl)
G1- normal saline	1.696 ± 0.02
G2- CCl4 (3mg/kg)	3.85 ± 0.155#
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	1.61 ± 0.004*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	2.13 ± 0.16*
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	1.69 ± 0.01*
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	1.89 ± 0.02*
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	2.58 ± 0.11*

Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.



Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

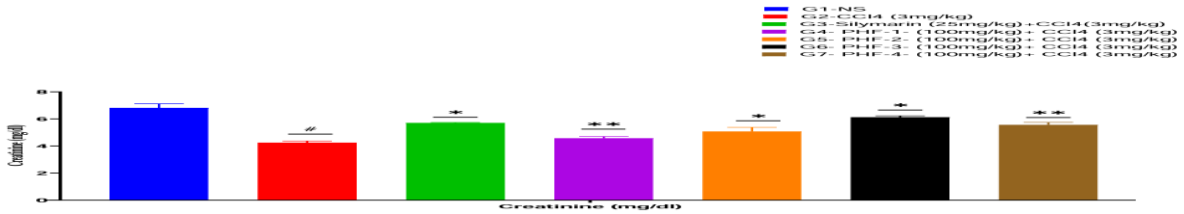
Figure no. 15: Effect of Treatments on Bilirubin

Creatinine (mg/dl): The assessment of kidney function was carried out by measuring serum creatinine levels. In the normal control group (G1), creatinine was observed at 6.82 ± 0.39 mg/dL. Interestingly, CCl4 administration in group G2 led to a significant decrease in creatinine levels to 4.25 ± 0.123 mg/dL, which is an unexpected result if CCl4 were causing kidney damage leading to accumulation. Typically, elevated creatinine indicates impaired kidney function. The decrease here might suggest an issue with muscle wasting or reduced creatinine production in severely ill animals, or it could be an artefact or reflect a complex physiological response in this specific CCl4 model.Silymarin treatment (G3) resulted in an increase in creatinine to 5.71 ± 0.03 mg/dL compared to the CCl4 group, moving it closer to the normal control, which is the desired direction if the CCl4 effect was a decrease. Among the nanoparticle formulations, PHF-3 (G6) demonstrated the most significant effect, bringing creatinine levels up to 6.13 ± 0.1 mg/dL, very close to the normal saline group. PHF-4 (G7) also showed a notable increase to 5.57 ± 0.189 mg/dL, followed by PHF-2 (G5) at 5.08 ± 0.3 mg/dL. PHF-1 (G4) had the least pronounced effect, with creatinine at 4.58 ± 0.129 mg/dL, still significantly higher than the CCl4 group but not as close to normal. Given the unexpected decrease in creatinine in the CCl4 group, the increase observed with silymarin and the various PHFs suggests a restoration towards normal physiological processes, which could include improved muscle integrity or overall metabolic recovery in the treated animals.

Table no. 16: Effect of Treatments on Cretinine

Group and Treatment	Creatinine (mg/dl)
G1- normal saline	6.82 ± 0.39
G2- CCl4 (3mg/kg)	4.25 ± 0.123 #
G3- Silymarin (25mg/kg)+CCl4(3mg/kg)	5.71 ± 0.03*
G4- PHF-1-(100mg/kg)+ CCl4 (3mg/kg)	4.58 ± 0.129**
G5- PHF-2-(100mg/kg)+ CCl4 (3mg/kg)	5.08 ± 0.3*
G6- PHF-3- (100mg/kg)+ CCl4 (3mg/kg)	6.13 ± 0.1*
G7- PHF-4- (100mg/kg)+ CCl4 (3mg/kg)	5.57 ± 0.189**

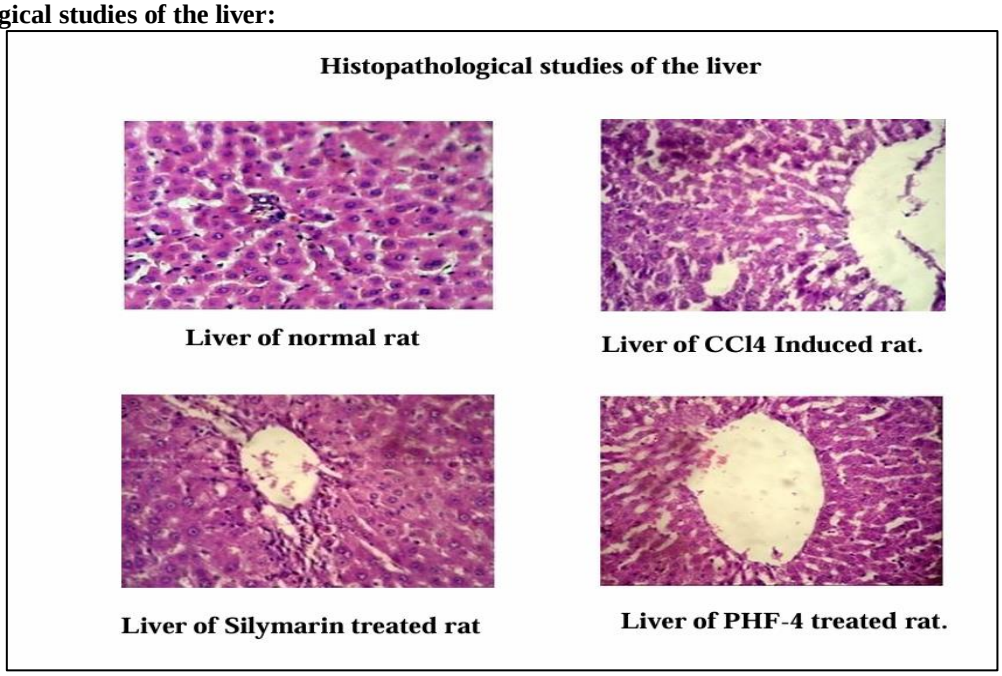
Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.



Dunnett's t test came after one way ANOVA. Mean±SEM, n=6 for all values. When compared to the vehicle-treated control group with toxic control, p<0.001; when compared to toxic control, p>0.05; *p<0.05; **p<0.01; ***p<0.001.

Table no. 16: Effect of Treatments on Creatinin

Histopathological studies of the liver:



CONCLUSION:

The plants was selected and authenticated by the Dr. Sadat.M. Quazi Associate Professor and Head of the Department of Botany, Mulana Azad College of Science, Arts and Commerce, Aurangabad. And all the three crude drug are extracted using the Hydro alcoholic solution (50:50) ratio for tinospora Cordifolia by cold maceration, 80% Alcohol for Tephrosia Perpuria and borhavia diffusia by hot maceration. The resultant extracts were evaluated for the various Phytochemical Constituent and the result is shown in Table no:02. The thin layer chromatograpy was performed using the different solvent and the extracts were compared with standard: Rutin. And percent yield were calculated as 10%, 10% and 20% for Tinospora Cordifolia, Tephrosia Perpuria and Borhavia diffusia respectively. Then this extract was used to prepared silver nanoparticles separately (Tinospora Cordifolia Silver Nanoparticle, Tephrosia Perpura Silver Nanoparticles, Borhevia Diffusia Nanopartiles). And then silver nanoparticles were prepared which contain the combination of all three extracts using Design Expert Software (Factorial). After applying the software Firstly, eight different formula. Using this formula we have formulate eight different batches of silver nanoparticles and evalution of the same was done. After feeding the obtain results in factorial design we got 71 solutions and then we selected one batch which has the desirability score is around 0.955 which is closer to 1. Hence, this batch was selected as the optimized batch. Characterization of all the nanoparticle was performed. Firstly, UV- Visible Spectroscopy was performed of each Nanoparticle. Then Prticle Size, SEM and TEM Image. The Invitro antioxidants was performed followed DPPH, Total Protein, SGOT, SGPT, Creatine, Birulliun and Histrology.

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