Preparation, Characterization And Evaluation Of Green Synthesis Nanoparticle Of Hydro Alcoholic Floret Extract Of Brassica Oleracea Var. Italica Plenck (Broccoli) Using Qbd Approach For Breast Tumor Cells T-47D Treatment

Ravindra B. Chintamani, Kishor S. Salunkhe, Amol R. Kharat, Sonali R. Chintamani, Rudra Pratap Singh, Machindra J. Chavan

Abstract : Background: Breast cancer is the second prime cause of death in women globally, and is expected to surpass heart diseases in the next few years. The resources available for diagnosis, prevention, and treatment of cancer are limited or non-existent. Unfortunately, presently available cancer chemotherapeutic agents surreptitiously affect the host cells of patients mainly bone marrow, epithelial tissues, reticule-endothelial systems and gonads. Metal nanoparticles have tremendous applications in the area of biomedical, agricultural, cancer, biotechnology and in other areas. Metallic NPs are commonly prepared by using various metals. In group of all metals, Silver has become the metal of researchers choice in treatment of cancer as a result of its solitary physiochemical properties. Objectives: The proposed study aimed to formulate the biologically synthesized green silver nanoparticles using floret extract of aerial part of Broccoli, wherein both silver as well as extract shows potential activity. Methods: Brassica oleracea var. Italica Plenck (Broccoli) hydro alcoholic floret extract mediated Silver Nanoparticles (Ag-Nps) were prepared by biological reduction method by implementing QbD approach. Resulted Ag-NPs were characterized for Morphology i.e. Particle Size and shape by FESEM, TEM and AFM. Other studies like Zeta Potential, % Yield, % Silver Loading and % Extract Loading were also undertaken, The studies also includes DSC, FTIR, UV-Spectroscopy, PXRD and EDS. Results: The studies showed promising results. In vitro and In vivo studies demonstrated that nanoparticles revealed higher anticancer efficacy than extract and proved stated hypothesis of significantly change in anticancer potential than individual. Conclusion: This study makes an attempt to overcome the limitations of conventional treatments of cancer and tumor with cost effective, eco-friendly, stable and safe targeted drug delivery as an alternative and / or complementary method of treatment.

Keywords: Breast Tumor Cell (T-47D), Green Synthesis Nanoparticle, Floret Extract of Broccoli, QbD Approach.

List of Abbreviations:
FE - Floret Extract; NPs – Nanoparticles; Ag-NP - Silver Nanoparticles; GSNPs Green Silver Nanoparticles; GSNP-F - Green Silver Nanoparticles of Florets; BNP -Blank Nanoparticles; CAM -Complementary and Alternative Medicine; CT - Chemotherapy; FTIR - Fourier Transform Infrared spectroscopy; NCCS - National Centre for Cell Science; MTT - (3-(4,5- Dimethylthiazol - 2- yl) - 2,5- Diphenyltetrazolium Bromide; MTT Assay - (3-(4,5- Dimethylthiazol - 2- yl) - 2,5- Diphenyltetrazolium Bromide Assay; PBS - Phosphate Buffered Saline; FE-SEM - Field Emission Scanning Electron Microscopy; HR-TEM - High Resolution Transmission Electron Microscopy; TGA - Thermo-Gravimetric Analysis; AFM - Atomic Forced Microscopy; XRD X-ray Diffraction; PXRD - Powder X-ray Diffraction; PSA - Particle Size Analysis; MP - Melting Point; BP - Boiling Point; UV-Vis - Ultra Violet Visible; DSC - Differential Scanning Colorimetry; SD - Standard Deviation; ICH - International Conference on Harmonization; IC_{50} - Inhibitory Concentration; QbD - Quality by Design; CQA - Critical Quality Attributes; TQPP / QTPP - Quality Target Product Profile; DoE - Design of Experimentation; ANOVA - Analysis of Variance, NDDS - Novel Drug Delivery System.

1. INTRODUCTION

Breast cancer is the second prime reason of cancer death in women globally, and is expected to surpass heart diseases in the next few years [1-2]. It reports for around seven % of worldwide burden of cancer and one-fifth of all the cancers in India [3]. As per American Cancer Society, a count of 29% incidences and 15% deaths due to breast cancer around the world has been anticipated [4]. In India, breast cancer was the leading cancer among females (24.85%) with the highest incidence and death rates being 10.53 and 16.18 %, respectively [5]. It has overtaken cervical cancer to become the leading cancer in Indian metro cities and is expected to double in 2016 [6]. It has been expected that by 2030, the universal occurrence of breast cancer would be grow to more than two million new cases per year; however, in India cases would reach up to two lakhs per year. Breast Cancer is a clinically diverse disease with multi-factorial etiology, triggered due to numerous risk factors comprising hormonal, genetic factors, environmental, dietary and lifestyle ; exposure to the ionizing radiation; as well as race, age, gender, ethnicity and history of family. Control of breast cancer is a foremost clinical challenge due to its complexity, heterogeneity and aggressiveness. The typical treatment available for breast cancer consists of chemotherapy, surgery, radiation therapy, targeted therapies and hormonal therapy. Even though these managements of cancer are highly efficacious, yet they are accompanying with grave side effects that have moved the global attention towards Complementary and Alternative Medicines (CAM). Use of CAM has become progressively common among the patients of breast cancer throughout the globe. It was investigated that the use of CAM in cancer patients differing from 7- 64% with increased use (47-83%) in breast cancer patients. Herbal medicine that forms an integral part of CAM has been testified to play a vital role in the management of breast cancer. Different medicinal plants including Taxus baccata (Pacific Yew), Podophyllum peltatum (Mayapple), Camptotheca acuminate (happy tree) and Vinca rosea (Periwinkle) have been evaluated in clinical trials for breast cancer [7]. Medicinal plants are a source of a large number of bioactive that are excellent anticancer agents as they have the efficacy to control the molecular mechanisms and various signaling pathways

implicated in carcinogenesis such as inflammation, oxidation, apoptosis, cell cycle, cell proliferation, metastasis, invasion and angiogenesis. Even though medicinal plants and their bioactive has been reported to be highly potent anticancer agents, the widespread use of herbal bioactives has been restricted due to their hydrophobic nature that reduces their bioavailability and reduces their therapeutic efficacy [8-9]. This problem has been overcome with the advent of nanotechnology, which has made a substantial impression on the development of novel drug delivery systems (NDDS) [10]. Lots of efforts have been undertaken to use modern nanotechnology to deliver herbal based drugs for safer and more effective treatment of breast cancer [11]. Nanotechnology is a promising technique holds a huge promise for the design and development of many types of novel products. Nanotechnology involves the tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired applications. Among them silver nanoparticles draw attention in cancer treatment due to its unique physical, chemical and biological properties. One of the emerging strategies has been the usage of herbal extracts for synthesizing metal nanoparticles (such as gold and silver) for anticancer applications [12]. Physical method, Chemical method and Biological method (Green synthesis) are the three major methods for synthesis of nanoparticle. Chemical approaches of synthesis are toxic and costlier. Thus, there is a rising need to develop eco-friendly processes, wherein no toxic chemicals are used in the synthesis. The amount of reduction of metal ions using herbal extracts has been established to be more rapidly as compared to micro-organisms and ensuring the development of stable metal nanoparticles. Broccoli is a cruciferous vegetable which affects the development of various types of cancers since it contains various chemical constituents such as, Selenium, Sulphoraphene, Glucosinolate and Diindolylmethane [13].

2. Materials and Methods

2.1 Experimental Materials

2.1.1. Procurement, Identification and Authentication

The Broccoli was received as gift from the local Farmer Shri. Kaka Kalbhor staying at Loni Kalbhor village near to Pune. The plant was recognized and validated from Botanical Survey of India, Western Regional Center, Pune, Maharashtra, India.

2.1.2. Experimental Chemicals

The organic solvents and other chemicals of analytical or chromatographic grade were purchased from Loba Chemie Pvt. Ltd., Mumbai and Merck Specialities Pvt. Ltd., Mumbai. Distilled water was utilized for the whole experiment.

2.1.3. Experimental Animal

Female Wistar Albino Rat (National Institute of Biosciences (NIB), Pune, India) weighing between 180 to 250 g was used. Housing of Experimental Animals under standard conditions of temperature, 12 h/12 h light/dark cycle and feed with standard pellet diet and tap water was done. The study protocol was placed before Institutional Animal Ethics Committee and approved vide letter number DYPIPSR/IAEC/17-18/P-20. All the experiments were approved and conducted as per the guidelines of local animal ethical committee. Acute oral toxicity was performed according to OECD-423 at dose 4000 mg/kg.

2.1.4. Experimental Cancer Cells

The cancer cell line T-47D was procured from the National Centre for Cell Sciences, Ganeshkhind, Pune-411007, Maharashtra, India. It was further sub cultured to perform the experimental study.

2.2 Preparation of Extract of Brassica oleracea var. Italica Plenck Floret

1000 gm of Floret of Brassica oleracea var. Italica Plenck were cut in to the small pieces and allowed for maceration with solvent mixture having ethanol and water (6:4) ratio for 7 days. After maceration filtration was carried out. It was then allowed for evaporation of solvent. After the evaporation, extract of Brassica oleracea var. Italica Plenck Floret (FE) was weighed for further biosynthesis of formulations.

2.3 Biosynthesis of Floret Loaded Silver Nanoparticles

Silver nanoparticles were prepared by green synthesis method using Brassica oleracea var. Italica Plenck Floret extract GSNP-F, along with silver nitrate solution by implementing QbD approach. 'Design Expert version 9' (State Ease, USA) software was used to generate design matrix and for purpose of statistical analysis. 2-Level Resolution V Factorial design generated 19 experimental runs with two levels and multiple coordinate points within two levels as shown in Table 1. The purpose of 2-Level Resolution V Factorial design was to optimize the factor settings, requiring greater precision in the estimated model. Polynomial equations were generated for each response particle size (Y1), Zeta Potential (Y2), Percent Yield (Y3), Silver Loading (Y4) and Extract Loading (Y5) of Silver nanoparticles by optimizing formulation and process parameter. Design space was constructed by setting the criteria for the CQAs using contour plots [14].

2.4 Characterization of Floret Loaded silver nanoparticles

2.4.1. Percentage Yield

Green Silver Nanoparticles (GSNP-F) were prepared and the percentage yield of nanoparticles was calculated. The percentage yield of Green Silver Nanoparticles were calculated from weight of dried nanoparticles recovered (W1) and sum of initial weight of starting material (W2) as by Eq. (1) [15].

$$\% \text{ Yield} = W_1/W_2 X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

Also, the reduction of the Ag+ ions by the supernatant of the test plant extracts in the solutions and formation of silver nanoparticles were characterized by UV-visible spectroscopy monitored by sampling the aqueous component (2.0 mL) and measuring the UV-VIS spectrum of solutions.

2.4.2. FT-IR Spectroscopy Study

FT-IR spectrum was used to identify the possible biomolecules responsible for the reduction of the Ag+ ions and capping of the bio-reduced Ag-NPs. The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55-Luminescence spectrometer). The dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method [16-20].

2.4.3. Differential Scanning Calorimetry

Thermal analysis was performed for the evaluation of the interaction between extract and Silver nitrate and the changes in the physical status of extracts before and after the manufacturing process. Differential scanning calorimetric (DSC) equipped with a thermal analysis data system (DSC 2920, TA Instruments, Alzenau, Germany). The instrument was calibrated using indium as the standard. The endothermic melting temperature for extract, Silver nitrate, physical mixture of extract and Silver nitrate and nanoparticles were determined with a DSC. 5 milligrams of samples were scanned from 20 °C to 160 °C at a rate of 10 °C/min [21-25].

2.4.4. Drug Loading

The entrapment efficiency (EE) of nanoparticles was calculated as follows in Eq. (2) [26].

Drug Loading (%) = Total amount of Drug – Free unentrapped Drug X 100 /Total amount of Drug

2.4.5. Particle Size Analysis

The Particle Size of NP's was determined using laser diffraction technique on Nanosizer 90ZS (Malvern Instruments, Southborough, MA) [27-29].

2.4.6. Zeta Potential

The sample was placed in transparent polystyrene corvette (path length = 1 cm) which was placed in the thermostatic sample chamber maintained at 25 ^{o}C by using Zeta meter, Delsa Nano. Zeta potential was measured automatically by the software [30-32].

2.4.7. Field Emmission Scanning Electron Microscopy (FE-SEM) and Energy Dispersive X-Ray Analysis (EDX)

To examine the sphericity and surface characteristics, the morphology study of nanoparticles were observed using scanning electron microscopy. The nanoparticles were coated with gold (<20 nm thick) using sputter (JFC-1100, JEOL, University of Pune) for EDX analysis of samples was done to check presence of silver loaded. FESEM is a microscope that works with electrons (particles with a negative charge) instead of light. These electrons are liberated by a field emission source. The object is scanned by electrons according to a zig-zag pattern. Electrons are liberated from a field emission source and accelerated in a high electrical field gradient. Within the high vacuum column these so-called primary electrons are focused and deflected by electronic lenses to produce a narrow scan beam that bombards the object. As a result, secondary electrons are emitted from each spot on the object. The angle and velocity of these secondary electrons relate to the surface structure of the object. A detector catches the secondary electrons and produces an electronic signal. This signal is amplified and transformed to a video scan-image that can be seen on a monitor or to a digital image that can be saved and processed further. EDX analysis of samples was done to check presence of silver loaded [33-34].

2.4.8. Transmission Electron Microscopy (TEM)

The morphology of Green Silver Nanoparticles was examined using an electronic transmission microscope (PHILIPS CM-200; London; operating voltages: 20-200 kv Resolution: 2.4 A^{o}). TEM images are formed using transmitted electrons (instead of the visible light) which can produce magnification details up to 1,000,000X with resolution better than 10 A^{o} [31, 35-38].

2.4.9. Atomic Force Microscopy (AFM)

In order to reveal the structure of the nanoparticles and their surroundings, optimized formulations FENP was examined on AFM instrument (Nano Surf Flex AFM, Easy Scan2) [39-43].

2.4.10. Powder X-Ray Diffraction (PXRD)

Sample was weighed and placed on the sample holder and smear uniformly onto a glass slide, assuring a flat upper surface. The PXRD patterns were recorded on an x-ray diffractometer (BRUKER aXS-D8 ADVANCE). The samples were irradiated with mono-chromatized CuKa radiation and analyzed between 2-80 ºC 2Ø. The patterns were collected with a voltage of 30kV and current of 30mA respectively. The scanning rate (2Ø/min^{-1}) was set at 10 ºC/mi. Samples of the aqueous solution of the silver nanoparticles (Ag-NPs) were prepared by centrifugation at 10,000 rpm for 30 min [21-25].

2.4.11. In vitro Drug Release Study

In-vitro release study of Brassica oleracea var Italica loaded silver nanoparticles were determined by dialysis bag diffusion method. In this method Dialysis Membrane – 50 (Hi-media) having molecular weight cut off 12000 to 14000 kD were used. Dialysis membrane was activated in boiling water for about 30 minutes. Then prepared silver nanoparticles were placed inside the dialysis membrane which is sealed at both the ends. Then it was placed in a beaker containing 100 ml phosphate buffer at pH 7.4. Then the beaker was placed over a magnetic Stirrer and rpm was maintained at 100. Samples (2 ml) were withdrawn at a definite time intervals of 1, 2, 3, 4, 5, 6, 12 and 24 h and replaced with equal amounts of fresh phosphate buffer solution pH 7.4. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer. Separate study was carried out by using PBS pH 5.2 as a solvent.

2.4.12. In vivo Evaluation of Anticancer Activity

2.4.12.1. Selection and maintenance of animals

Wistar Albino Rat (National institute of Biosciences, Pune, India) weighing between 180 to 250 g of either sex was used. Animals were housed under standard conditions of temperature, 12 h/12 h light/dark cycle and fed with standard pellet diet and tap water. The protocol (DYPIPSR/IAEC/17-18/P-20) was approved by Institutional Animal Ethics Committee. All the experiments were

approved and conducted as per the guidelines of local animal ethical committee.

2.4.12.1.1. Acute Toxicity Study in Rat

The oral acute toxicity study of extracts and formulations were evaluated according to Organization for Economic Cooperation and Development (OECD) guideline 423 on Wistar Albino Rat (200–300 g), where the limit test dose of 4000 mg/kg was used. All the animals were kept at overnight fasting before to every experiment with free excess to water. The animals were divided into four groups, each comprising 6 animals. The 1st group was served as a control, while 2nd, 3rd, 4th, and 5th groups were considered as test groups, received orally (dissolved in normal saline) extracts and formulations (dissolved in normal saline) at dose of 300 mg/kg, 2000 mg/kg and 4000 mg/kg. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The animals were observed for any toxic effect for first 4 h after the treatment period. Further animals were investigated for a period of 3 days for any toxic effect. Behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, constipations, changes in eye and skin colors, etc. were observed [44].

2.4.12.1.2. Preparation of standard drug for animals dosing

Paclitaxel was used as standard drug at a dose of 0.2 mg/kg orally for the evaluation of anticancer activity. The animals were divided in to 6 groups consisting 6 animals in each group.

2.4.12.1.3. Murine Tumour Model [45-46]

a. Experimental animals

1. The standard pellet diet composing 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamin, and 55% nitrogen-free extract (carbohydrates) was used for feeding.
2. The rats were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycle.

b. Preparation of Tumour cells T-47D

1. Cells were grown in complete medium and excluded any contamination.
2. When cells were 70-80% confluent, 3-4 h before harvesting, replaced medium with fresh medium to remove dead and detached cells.
3. Removed medium and washed cells with PBS. Added a minimum amount of trypsin-EDTA. Dispersed cells and added complete medium (10:1 to 5:1).

Centrifuged immediately at or below 1500 rpm for 2-5 min and washed twice with PBS and stored cells on cell.

4. Counted cells using a hemocytometer. Using trypan blue staining to exclude dead cells.
5. Mixed cells 1:1 with trypan blue solution (Trypan Blue: dilute at 0.8 mM in PBS. Stored at room temperature and kept for 1 month.). Viable cells exclude trypan blue, while dead cells stain blue due to trypan blue uptake.
6. Cells were suspended in a volume so that 300 µl contains required number of cells per injection. Usually, $1x10^7$ cells are needed per injection.

c. Procedure for the injection into the rats

1. Rats were inoculated with estradiol-17β (1.7 mg/pellet) before an injection of 1×10^7 T-47D breast cancer cells subcutaneously into inguinal region of mammary fat pad.
2. The tumor size observed and measured in three dimensions with calipers every 2 days starting at day 7.
3. Rats were observed for any change in behaviour, appearance or weight.
4. After confirmation of tumour induction by histopathology treatment was started.
5. Standard and Test samples were injected in to Animals according to schedule.
6. The increase in life span of drug treated rats was calculated and compared with untreated tumor bearing animals.
7. The life span of experimental animals was monitored and calculated by using the formula ((T − C)/C) × 100, where 'T' indicates the number of days the treated animals survived and 'C' indicates that number of days that tumor animals survived.

d. Evaluation of Parameters [47-49]

1. Cage side observations

For the acute toxicity study animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours (with special attention given during the first 4 hours.

2. Feed and water consumption

The amount of feed and water consumed for 24 h was measured weekly from the quantity of feed and water supplied and the amount remaining after 24 h for till the end of the experiment.

3. Body weight Determination

Individual animal body weight was recorded weekly till the end of the experiment.

4. Tumor Size

Animals were dissected and tumours were isolated. Tumor size was measure by Vernier Calliper.

5. Tumor Weight

Tumor weight was measured by isolating tumours after dissecting Animals.

6. Hematological Parameters

Blood of all experimental animals was collected by retro orbital method before cell line injection, after cell line injection and completion of treatment and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell count (WBC). Comparison was made among all groups. Blood was collected immediately into EDTA bulbs for analysis of hematological parameters.

7. Statistical analysis

The results are expressed as mean + SEM (n=6). Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. Analysis of variance (ANOVA) was used to compare the mean value of data and $P<0.05$ were considered as significant.

3. Result and Discussion

3.1. Biosynthesis of Silver Nanoparticles of Plant

Silver nanoparticles were prepared by green synthesis method using Brassica oleracea var. Italica Plenck Floret extract along with silver nitrate solution. Visual examination of synthesized silver nanoparticles for the color change

from yellow to brown indicates the formation of silver nanoparticles as shown in Figure 1. This was further confirmed by Uv- visible spectral analysis of the sample at different time intervals and found to 411.27 nm for Floret nanoparticles (Figure 2).

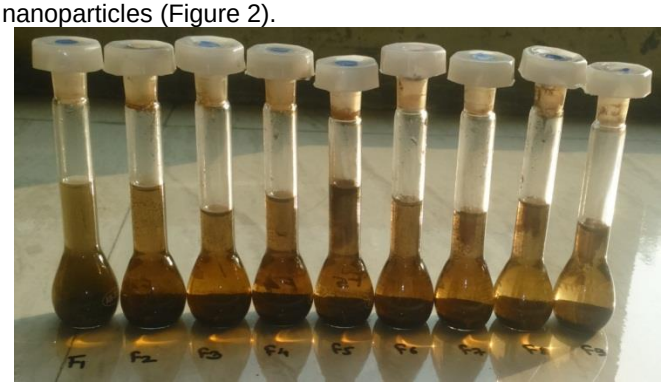

Figure 1: *Confirmation of Formation of Broccoli Floret Extract Loaded Silver Nanoparticles*

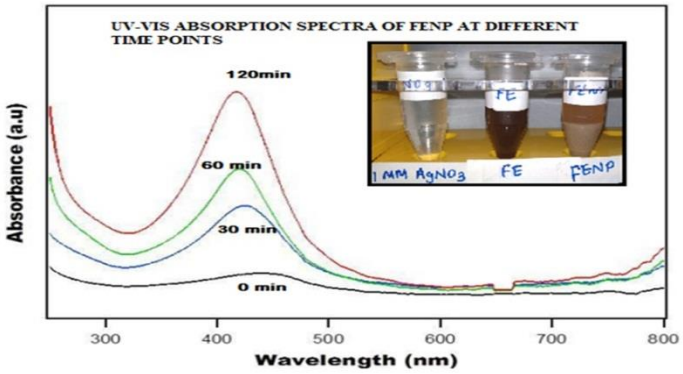

Figure 2: *UV- Visible absorption spectra of Brassica oleracea var. Italica Plenck Floret Extract Mediated Silver Nanoparticles at different time intervals*

To develop the robust formulation of GSNP-F Nanoparticles, we need to conduct the design of experimentation by taking the high risk parameters as critical process and material attributes and by selecting suitable statistical model we need to perform the DoE study to see the effect on the CQAs and to achieve desirable QTPP. Therefore these high risk components i.e. concentration of Silver, concentration of Extract, stirring speed, Reaction Time and pH were selected to conduct design of experimentation (DoE) to see the effect on CQAs; particle size, particle shape, Zeta Potential, % Yield, Silver Loading and % Extract Loading. Different criteria's for selection of statistical model to conduct design of experimentation (DoE) were as follows,

- The nature of the problem and/or study (e.g., a screening, optimization, or robustness study).
- Type of study i.e. main effects or two-way interactions.
- Number of parameters, the factors and interactions to be studied (e.g., two, three, four, six, or nine factors.
- Number of levels of parameters.
- Available resources (e.g., time, labor, cost, and materials).

By considering the all above criteria's, we selected '2-Level Resolution V Factorial design' for conducting the experimental study. '2-Level Resolution V Factorial design'' is factorial design which can be used for screening as well as optimization purpose for process as well as formulation parameters. It has better ability to identify multiple factor interactions along with main effect on the selected response as compared to full factorial and fractional factorial designs. Resolution V design can evaluate main effects and 2 FI, whereas 2 FI may aliases with other 2 FI, considering the purpose of study, this design was selected). 'Factorial Randomized- Resolution V design' also has ability to provide the curvature effect of selected high risk parameters due to inclusion of center points in the design. GSNP-F nanoparticles were prepared by Biological Reduction method and the compositions were as per the formulation. Design space (overlay plot) by overlapping contour plot of all CQAs (responses) were constructed shown in Figure 3, characterizes acceptable ranges of the concentration of Silver (A or X_1), concentration of Extract (B or X_2), stirring speed(C or X_3), Reaction Time (D or X_4) and pH (E or X_5) which provides assurance that CQAs will be within acceptable criteria. Robustness can be assured of nanoparticles formulation if operated in the range of design space. Validation of the applied model was carried out by performing the 3 formulations with combinations of factors within obtained design space and predicted and observed results were compared for particle size, Zeta Potential, %

Table 1: *Formulation of Floret Extract Loaded Green Silver NPs using 2-Level Resolution V Factorial design*

Run	Factors: Process and Formulation variables					CQAs Response					
	A Conc. of $AgNO_3$ (mM)	B Conc. of Floret Extract (gm)	C pH	D Reac Tme (hr)	E Stirring Speed (rpm)	Y_1: PSA (nm)	Y_2: Zeta P (mV)	PDI	Y_3: % Yield	% Loading* Y_4: SN	% Loading* Y_5: Extract
1	1	0.5	4	4	250	40.15	-10.35	0.210	21.22	24.15	52.25
2	3	0.5	4	4	150	35.20	-11.45	0.532	18.30	31.38	55.38
3	1	1.5	4	4	150	57.30	-46.76	0.532	24.12	27.16	55.54
4	3	1.5	4	4	250	64.21	-40.65	0.278	26.42	36.04	64.16
5	1	0.5	12	4	150	29.15	-7.89	0.746	16.01	29.25	56.27
6	3	0.5	12	4	250	26.36	-8.0	0.159	17.22	30.38	54.15
7	1	1.5	12	4	250	48.18	-40.5	48.18	28.18	27.08	58.39
8	3	1.5	12	4	150	64.45	-40.34	64.45	24.39	33.28	67.46
9	1	0.5	4	12	150	30.12	-25.7	30.12	14.15	26.01	53.15
10	3	0.5	4	12	250	28.20	-9.86	28.20	15.34	32.15	56.28
11	1	1.5	4	12	250	62.30	-47.65	62.30	21.02	26.33	46.45
12	3	1.5	4	12	150	63.17	-39	63.17	23.24	28.09	58.15
13	1	0.5	12	12	250	34.31	-10.43	34.31	16.31	23.44	54.20
14	3	0.5	12	12	150	41.80	-14.5	41.80	19.54	25.15	58.23
15	1	1.5	12	12	150	63.01	-39.78	63.01	24.05	24.55	54.16
16	3	1.5	12	12	250	74.35	-40.67	74.35	26.31	36.15	56.34
17	2	1	8	8	200	33.40	-27.87	33.40	32.28	23.05	72.21
18	2	1	8	8	200	32.22	-28.65	32.22	31.25	24.64	69.08
19	2	1	8	8	200	29.15	-32.45	29.15	28.32	21.41	68.12
GSNP-F-1						43.00	-22.5	0.332	20.00	28.90	56.00
GSNP-F-2						48.00	-26.84	0.365	20.20	29.50	55.90
GSNP-F-3						48.50	-27.2	0.398	20.50	28.0	56.00

yield, % Silver loading, % Extract loading and obtained results were compared with predicted results as shown in Table 1 . The composition of the checkpoints formulation, the predicted and obtained response values of all the response variables, and the percentage error in prognosis. Thus, the low magnitudes of error in the current study indicated a high prognostic ability of GSNP-F nanoparticles formulations using 2-Level Resolution V Factorial design. The acceptance range for CMA/CPP is given in Table 2.

Table 2: *Acceptance range for CMA/CPP*

Acceptance Range for CMA/CPP to achieve CQA within Acceptance criteria		Acceptance Range	
Conc. of $AgNO_3$	1.7- to 2.65 mM	Particle Size	37 – 57 nm
Conc. of Extract	0.65 to 1.38 ml	Zeta Potential	-15 to -38 meV
pH	8	% yield	18 – 23 %
Reaction Time	8 hr	Silver Loading	28 – 29 %
Stirring Speed	200 rpm	Extract Loading	55 – 56 %

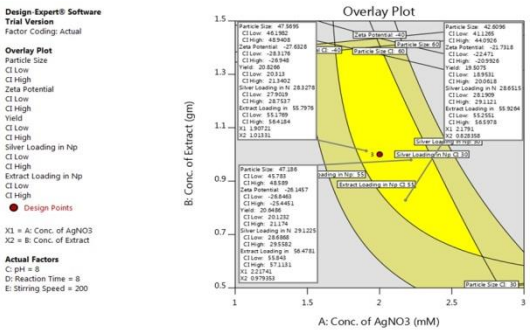

Figure 3: *Functional design space for Broccoli Floret mediated Silver NPs*

3.2. Characterizations of Floret Loaded Silver Nanoparticles

3.2.1. FT-IR Spectroscopy Study

FT-IR spectrum of Silver Nitrate, Broccoli Floret Extract and optimized formulation was taken as shown in Figure 4. FTIR spectrum was carried out in the wavelength region between 500 to 4000 cm-1 to identify the biomolecules for capping and efficient stabilization of the nanoparticles of the metal Nps. In the FTIR spectrum, sharp absorption peaks at 1388.5 cm^{-1} indicates C=C stretching of α, β, unsaturated ketone, and absorption peaks at 825.384 cm^{-1}, 802.242 cm^{-1} and 732.817 cm^{-1} specifies the presence of alkanes, alkenes, and aromatic rings. In the FTIR a spectrum of band between 3289.96 cm^{-1} corresponds to CH stretching and NH stretching. Peaks at 1762.62 indicate C=O stretching in Carboxylic acid. Groups at 2062.5 cm^{-1}indicates C=C(S) of alkane which is important for bioreduction and capping of NPs. FT-IR spectrum of optimized formulation shows peaks at 3278.39 cm^{-1} (strong O-H bonding) which indicates the presence of O-H stretching of carboxyl group. Further, the peaks observed at 3074.94 cm^{-1}; represents the C-H stretching bonds of alkanes. The peak observed at 1456.96 cm^{-1}, 1542.77 cm^{-1} and 2359.48 cm^{-1} indicates C=C aromatic conjugates. The sharp peak at 1162.87 cm^{-1} and 1286.29 cm^{-1}; are assigned to C-N stretching vibrations of proteins. The positions of these bands are close to that reported for native proteins. The sharp peaks at 2359.48 cm^{-1}, 1716.34 cm^{-1}, 1384.64 cm^{-1} and 1062.59 cm^{-1} which showed that the biologically synthesized Ag-Nps were capped and stabilized by proteins. It was found that hydroxyl, carboxyl groups and phenolic group were in spectrum which are responsible for reducing and stabilizing.

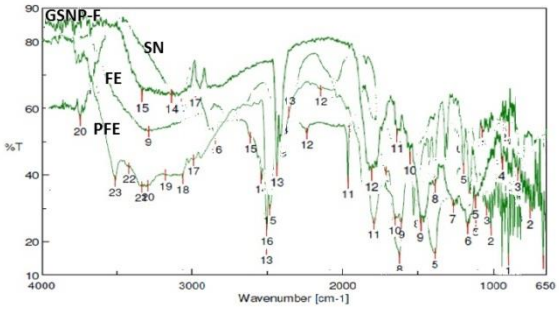

Figure 4: *FTIR Overlay Spectra of GSNP-F*

3.2.2. Differential Scanning Calorimetry

The isothermal behavior of Ag-NPs has been investigated using DSC technique over a temp range of 40 ^{0}C to 300 ^{0}C in ambient air. Figure 5 shows DSC Curve of Ag-NPs exhibiting the low temp broad endothermic peak at 71.97 ^{0}C due to loss of water molecule adsorbed on surface of Ag-NPs during synthesis conditions. Disappearance of major endothermic peak indicating formation of NPs with uniform distribution of extract in Silver confirmed by endothermic peak at 134.80 ^{0}C. Figure 5, shows Overlay DSC thermogram of Silver Nitrate, Floret Extract, Physical mixture of Silver Nitrate and Floret Extract and optimized formulation.

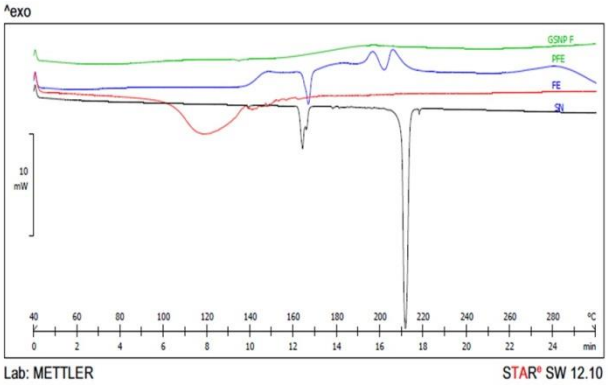

Figure 5: *Overlay Graph of Differential Scanning Calorimetry of GSNP-F*

In the DSC thermogram of the Silver Nitrate, exhibited a sharp endothermic peak at 210 ^{0}C. The reported M.P. through analysis is closely related to metallic Silver. The type of peak highlighting the working behavior which may indicate the crystalline nature. The Physical Mixture of Silver Nitrate and Floret Extract exhibited a sharp endothermic peak at its Melting Point at 166.72 ^{0}C. The peak values obtained were slightly shifted from the standard peak values of excipients.

3.2.3. Particle Size and Zeta Potential

Particle size is analyzed by using zeta sizer and the average particle size was found to be 46.7 nm for GSNP-F. Zeta potential measured was found to be -25.43 mV for GSNP-F. These values indicate the stabilization of silver nanoparticles.

3.2.4. Percentage Yield and Extract Loading

The percent yield was found to be 20 % w/w for GSNP-F. The % Silver loading in GSNP-F was found to be 28.46 %. % Extract loading in GSNP-F was found to be 56.5 %.

3.2.5. Powder X-Ray Diffraction (PXRD)

The crystalline nature of optimized formulations GSNP-F was confirmed by XRD. Figure 6, shows the XRD pattern of optimized formulations GSNP-F. Diffraction peaks were observed in the 2Θ range 20° to 80°. It showed optimized formulations GSNP-F is found to be crystalline in nature. A number of Bragg Reflections corresponding to (111), (200), (220) and (311) sets of lattice planes are observed, which can be indexed to face-centered cubic Silver. In XRD pattern of optimized formulation GSNP-F, shows intense diffraction peak at 2Θ= 38.08°, 44.26°, 64.42° and 77.36°. The intense peak at 38.08° indicates high degree of Crystallanity.

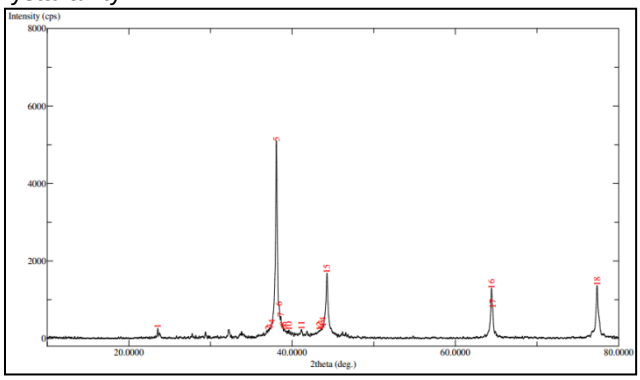

Figure 6: *XRD Pattern of GSNP-F*

3.2.6. Morphology of GSNP-F Nanoparticle

3.2.6.1. FE-SEM: Field Emission Scanning Electron Microscopy (FE-SEM) provided the morphology and size details of optimized formulations GSNP-F. The obtained results showed that the average diameter was found to be 40.38 nm and the shape was found to be spherical as shown in Figure 7. Irregularly-shaped nanoparticles will tend to agglomerate easily. The bond energy of spherical nanoparticle is greater than other shaped nanoparticles hence improves efficiency.

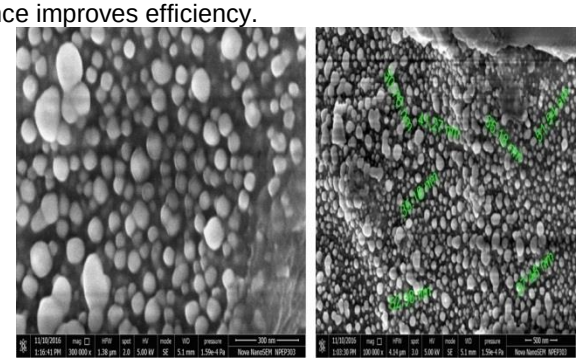

Figure 7: *FE-SEM Image of GSNP-F*

3.2.6.2. EDS

Energy Dispersive X-Ray Analysis (EDAX) was performed for Broccoli Floret Extracts as well as for Optimized formulations GSNP-F. Broccoli Floret Extract shows presence of S, K, Cl, O, C and N Elements. The presence of silver in optimized formulations GSNP-F was confirmed with the help of EDAX. The EDAX pattern in Figure 8, clearly shows that Silver Nanoparticles formed by the reduction of Silver ions using Broccoli Floret Extracts are crystalline in nature. The EDAX pattern of optimized formulations showed a peak at 3KeV confirming the presence of metallic Silver. Some elements like C, O, N, S and K were observed which may be a contribution of Plant extracts. It also confirms the Silver Weight % in GSNP-F i.e. 67. 65 wt % as shown in Table 3. Hence EDX showed predominating quantity of Silver as an evidence of nano bio metallic formation.

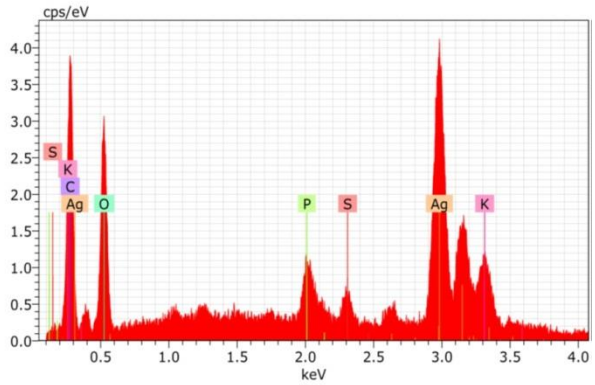

Figure 8*: EDS of GSNP-F*

Table 3*: EDS report of GSNP-F*

El	AN	Series	Unn. C [wt.%]	Norm. C [wt.%]	Atom. C [at.%]	(1 Sigma) [wt.%]
Ag	47	L-Series	50.31	67.65	27.11	1.76
O	8	K-Series	10.72	14.42	38.96	1.71
K	19	K-Series	6.02	8.09	8.94	0.27
C	6	K-Series	3.83	5.15	18.53	0.64
P	15	K-Series	2.07	2.78	3.88	0.13
S	16	K-Series	1.42	1.91	2.57	0.10
Pt	78	M-Series	0.00	0.00	0.00	0.00
		Total	74.92	100.00	100.00	

3.2.6.3. TRANSMISSION ELECTRON MICROSCOPY (TEM)

Morphology and particle size of optimized formulations GSNP-F was characterized by using HR-TEM analysis. It reveals that the AgNPs synthesized with Floret extract of Broccoli (GSNP-F) shown in Figure 9 was predominantly nanosized and spherical in shape with a diameter ranging from 25 nm to 60 nm. The capping of Silver nanoparticles by biocomponents from Broccoli floret extract is visual from

the pictures. The Selected Area Electron Diffraction (SAED) pattern for GSNP-F shown in Figure 9, exhibits concentric rings confirms that the Nps are highly crystalline in nature.

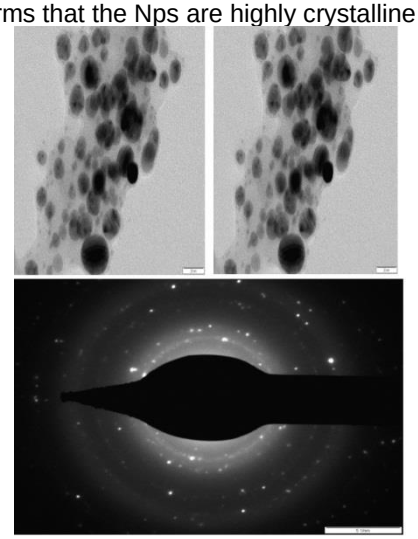

Figure 9: *TEM and SAED Image of GSNP-F*

3.2.6.4. AFM

In order to reveal the structure of the nanoparticles and their surroundings, optimized formulations GSNP-F was examined under Atomic Forced Microscopy. Figure 10(a) shows 2 D and Figure 10(b), Figure 10(c), Figure 10(d) shows 3D images respectively of the silver nanoparticles. Figure 10(e) and Figure 10(f) shows AFM Histogram and AFM Peak Distribution of GSNP-F respectively. It was noted that the particles were of uniform size associated with phytochemicals of the extract. The average size of particles of optimized formulations GSNP-F was measured between 25 nm to 60 nm. The magnified 3D image revealing the clear structures of optimized formulation of GSNP-F. 3D image of single individual particles were shown. The Ag nanoparticle is seen at the center surrounded by a thick layer of phytochemicals of the extract, with a deep circular canal like structure in between. The boundary is leaching at different places looking like a serrated circular layer. The presence of, poorly soluble and insoluble chemical components in the Broccoli Floret extract may have caused the formation of this unique structure; the poorly soluble components, being partially soluble, might cause the leaching and depression, while insoluble components might cause the formation of stable boundary around the particle thereby controlling its growth, and in turn, giving rise to uniform sized Ag nanoparticles.

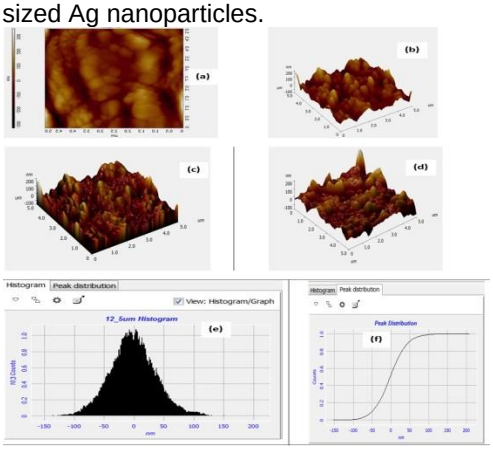

Figure 10: *Images of GSNP-F (a) 2D AFM, (b); (c); (d) 3D AFM, (e) AFM Histogram, (f) AFM Peak Distribution*

SEM, TEM and AFM analysis was carried out to study the morphology of synthesized Brassica oleracea var. Italica Plenck Floret and Leaves extract silver nanoparticles and found to be spherical in shape and average particle size of GSNP-F and GSNP-L was found to be 40.38nm and 55.92 nm respectively. EDX analysis revealed the presence of silver and functional groups of extract confirmed the formation of Brassica oleracea var. Italica Plenck Floret and Leaves extract loaded silver nanoparticles. DSC analysis confirmed the formation of Silver nanoparticles.

3.2.7. In-Vitro drug release

In- vitro drug release study was carried out using dialysis bag diffusion method and % cumulative drug released at different time intervals (1h, 2h, 3h, 4h, 5, 6h, 12 h and 24 h) was calculated. Figure 11(a), 11(b) and 11(c) summarized the % Cumulative release of Brassica oleracea var. Italica Plenck floret mediated silver nanoparticles by using Phosphate Buffer Solution pH 7.4 at wavelength 411 nm. Study of 3 optimized batches was carried on 6 units. Data shows that there is linear release from 1 h to 12 h, whereas burst release was observed at 24 h.

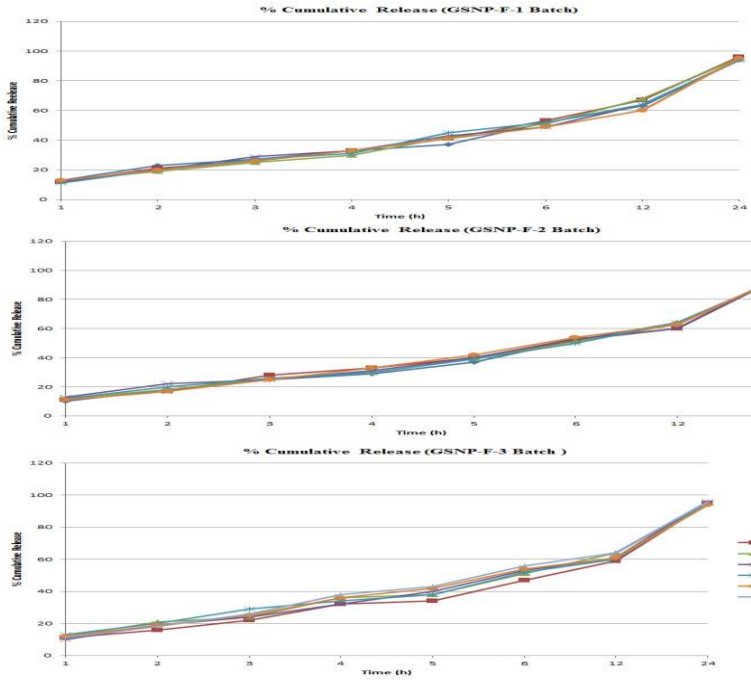

Figure 11: *% Cumulative Drug release of (a) GSNP-F-1, (b) GSNP-2, (c) GSNP-3 in PBS pH 7.4*

The in-vitro drug release studies were evaluated. From the release studies it was found that Brassica oleracea var. Italica Plenck Floret extract mediated silver nanoparticles follows Burst release after 24 h.

3.2.8. In-Vivo Evaluation

3.2.8.1. Acute Toxicity Study

Acute toxicity studies conducted as per the internationally accepted protocol drawn under the OECD guidelines 423 in Wistar Rat. The acute toxic effect of extracts and

formulations were determined as per the OECD guideline 423, where the limit test dose of 4000 mg/kg was used. No treatment related toxic symptom or mortality were observed after oral administration of the test plant extracts and formulations at a dose of 300, 2000 and 4000 mg/kg. The general behavioral of the treated animals and control group was observed first for short period (4 h) followed by long period (72 h), did not display any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Therefore, the extracts and formulations seems to be safe at a dose level of 4000 mg/kg, and the Maximum Tolerable Dose (MTD) (formerly known as LD50) was considered be >4000 mg/kg. However, there were sign of sedation, and drowsiness after the administration of formulations at dose of 4000 mg/kg compared to control group. The parameters observed for acute toxicity study after the administration of the test plant extracts and formulations compared with normal group are presented in Table 4. General appearance and behavioral observations of acute studies demonstrated no significant effect on Digestion, Temperature, Food Intake, Urination, Rate of Respiration, change in Skin, Sedation, Eye Color, Diarrhea, Coma, and Death. FENP and BNP showed drowsiness and Sedation as demonstrated in Table 5.

Table 4: *Acute Toxicity Study*

Sr.no.	Parameters	Observations
1	Body position	Normal
2	Locomotion	Normal
3	Rearing	Normal
4	Respiration	Normal
5	Righting reflex	Normal
6	Lacrimation	Normal
7	Alertness, reactivity to touch stimuli	Normal

Table 5*: General appearance and behavioral observations of acute toxicity study*

Sr. No	Observation	Dose mg/kg	Control Group	FE	FENP	BNP
1	Digestion	300	300	NO	NO	NO
		2000	2000	NO	NO	NO
		4000	4000	NO	NO	NO
2	Temperature	300	Normal	No Change	No Change	No Change
		2000	Normal	No Change	No Change	No Change
		4000	Normal	No Change	No Change	No Change
3	Food Intake	300	Normal	Normal	Normal	Normal
		2000	Normal	Normal	Normal	Normal
		4000	Normal	Normal	Normal	Normal
4	Urination	300	Normal	Normal	Normal	Normal
		2000	Normal	Normal	Normal	Normal
		4000	Normal	Normal	Normal	Normal
5	Rate of Respiration	300	Normal	No Effect	No Effect	No Effect
		2000	Normal	No Effect	No Effect	No Effect
		4000	Normal	No Effect	No Effect	No Effect
6	Change In Skin	300	No Effect	No Effect	No Effect	No Effect
		2000	No Effect	No Effect	No Effect	No Effect
		4000	No Effect	No Effect	No Effect	No Effect
7	Drowsiness	300	Not Present	No Effect	No Effect	No Effect
		2000	Not Present	No Effect	No Effect	No Effect
		4000	Not Present	No Effect	Present	Present
8	Sedation	300	No Effect	Not Present	Not Present	Not Present
		2000	No Effect	Not Present	Not Present	Not Present
		4000	No Effect	Not Present	Observed	Observed
9	Eye Color	300	No Effect	No Effect	No Effect	No Effect
		2000	No Effect	No Effect	No Effect	No Effect
		4000	No Effect	No Effect	No Effect	No Effect
10	Diarrhea	300	Not Present	No Effect	No Effect	No Effect
		2000	Not Present	No Effect	No Effect	No Effect
		4000	Not Present	No Effect	No Effect	No Effect
11	Coma	300	Not Present	Not Present	Not Present	Not Present
		2000	Not Present	Not Present	Not Present	Not Present
		4000	Not Present	Not Present	Not Present	Not Present
12	Death	300	Alive	Alive	Alive	Alive

Sr. No	Observation	Dose mg/kg	Control Group	FE	FENP	BNP
		2000	Alive	Alive	Alive	Alive
		4000	Alive	Alive	Alive	Alive

Where, FE – Floret extract; FENP – Floret extract loaded nanoparticle; BNP – Blank nanoparticle

3.2.8.2. Murine Tumour Model

Murine tumour model was selected to determine the anticancer potential of extracts and formulations. Wistar rat Animals were selected and divided in to six groups containing 6 animals in each group. T-47D cell line was selected to induce the cancer. After the cell line treatment, confirmation of Tumour formation was done by Histopathology. One animal from each group was sacrificed and breast was isolated. The isolated breast was sent to Chaitanya Laboratories to undertake the histopathology for confirmation of tumour. Studies noted multifocal mild neutrophilic/lymphocytic infiltration and multifocal mild neovascularization at dermis and subcutis remarked as Mild inflammation with neovascularization. Large tracts of tumor cells with little stroma with fibrous tissue at subcutis was observed. Diffuse nuclear pleomorphism (variations in nuclear size and staining), without evidence of gland formation was noted. Increased nuclear cytoplasm ratio with eosinophilic cytoplasm is seen. Mitotic figures are observed (1-2/3 hpf) Multifocal moderate lymphocytic infiltration at tumor area (2+) Multifocal moderate neovascularization (3+) indicated Low grade Solid Mammary Adenocarcinoma as shown in Figure 12(a), (b), (c), (d) and (e).

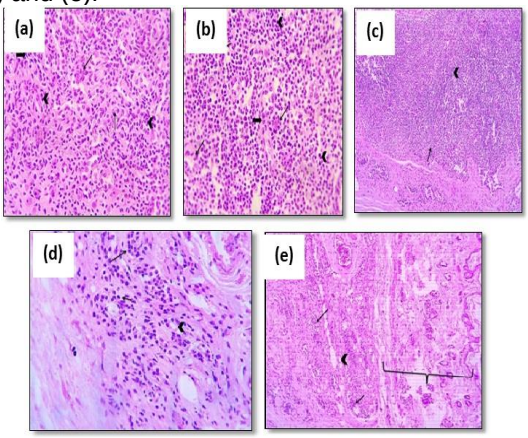

Figure 12*: (a) Showing neutrophilic /lymphocytic infiltration (arrow) and neovasularization (arrow head). Note normal histology of skin (brace), (b); (c); (d) Showing neutrophilic / lymphocytic infiltration (arrow) and neovascularization (arrow head), (e) Showing complete section of solid tumor at subcutis (arrow) and note prominent lymphocyte infiltration at tumor area (arrowhead) (H & E, 100X)*

3.2.8.3. Effect on Treated Animal

3.2.8.3.1. Feed Consumption

In cancer, Anorexia is observed due to metabolic changes and of progressive undernourishment. Results (Figure 13(a), showed that there was significant (P<0.001) decrease in the feed consumption in NC group as compared to PC group showing the decline in the appetite due to induction of tumor in NC group. STD, BNP, FE, FENP, showed significant (P<0.001) increase in the feed consumption as compared to NC showing the normalization of the feed consumption by all the test groups. FENP showed significant (P<0.001) increase in the feed consumption as compared to BNP and FE group showing more potent activity of FENP as compared to BNP and FE. When FE groups were compared with STD groups, no significant different was found.

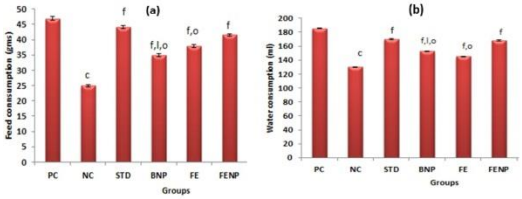

Figure 13: *Effect on (a) Feed Consumption, (b) Water Consumption*

The results are expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. NC compared to PC: a-P<0.05, b-P<0.01, c-P<0.001; all treatment groups compared to NC: d-P<0.05, e-P<0.01, f-P<0.001; FENP compared to STD: g-P<0.05, h-P<0.01, i-P<0.001; BNP and FE compared to FENP: j-P<0.05, k-P<0.01, l-P<0.001

3.2.8.3.2. Water Consumption

Cancer can lead to small changes in blood sugar levels that cause decrease in thirst. Results (Figure 13(b), showed that there was significant (P<0.001) decrease in the water consumption in NC group as compared to PC group showing the decline in the thirst due to induction of tumor in NC group. STD, BNP, FE, FENP, showed significant (P<0.001) increase in the water consumption as compared to NC showing the normalization of the water consumption by all the test groups. FENP also showed significant (P<0.001) increase in the water consumption as compared to BNP and FE group showing more potent activity of FENP as compared to BNP and FE. When FE groups were compared with STD groups, no significant different was found.

3.2.8.3.3. Body weight Determination

Cachexia, weight loss is associated with a marked decrease of food intake and severe alteration of body composition that leads to a negative protein and energy balance. Results (Figure 14), showed that there was significant (P<0.001) decrease in the body weight in NC group as compared to PC group showing the loss in the body mass due to induction of tumor in NC group. STD, BNP, FE, FENP showed significant (P<0.001) decrease in the body weights as compared to NC showing the normalization of the body weights by all the test groups. FENP showed significant (P<0.001) increase in the body weights as compared to BNP and FE group showing more potent activity of FENP as compared to BNP and FE.

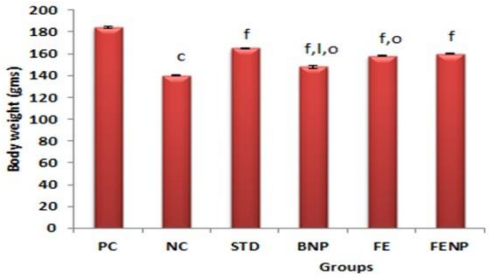

Figure 14: *Effect on Body Weight*

The results are expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. NC compared to PC: a-P<0.05, b-P<0.01, c-P<0.001; all treatment groups compared to NC: d-P<0.05, e-P<0.01, f-P<0.001; FENP compared to STD: g-P<0.05, h-P<0.01, i-P<0.001; BNP and FE compared to FENP: j-P<0.05, k-P<0.01, l-P<0.001

3.2.8.3.4. Tumor Size

Results (Figure 15(a), showed that there was significant (P<0.001) increase in the tumor size in NC group as compared to PC group (with no tumor) showing the induction of tumor in NC group. STD, BNP, FE, FENP group (test groups) showed significant (P<0.001) decrease in the tumor size as compared to NC group showing potential anti-tumor activities by all the test groups. FENP showed significant (P<0.001) decrease in the tumor size as compared to BNP and FE group showing more potent anti-tumor activity of FENP as compared to BNP and FE. When FE groups were compared with STD groups, no significant different was found.

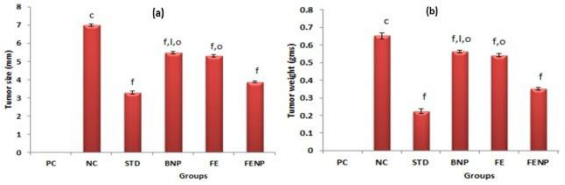

Figure 15*: Effect on (a) Tumor Size, (b) Tumor Weight*

The results are expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. NC compared to PC: a-P<0.05, b-P<0.01, c-P<0.001; all treatment groups compared to NC: d-P<0.05, e-P<0.01, f-P<0.001; FENP compared to STD: g-P<0.05, h-P<0.01, i-P<0.001; BNP and FE compared to FENP: j-P<0.05, k-P<0.01, l-P<0.001.

3.2.8.3.5. Tumor Weight

Breast cancer occurs when some breast cells begin to grow abnormally. These cells divide more rapidly than healthy cells do and continue to accumulate, forming a lump or mass. Results (Figure 15(b), showed that there was significant (P<0.001) increase in the tumor weight in NC group as compared to PC group (with no tumor) showing the induction of tumor in NC group. STD, BNP, FE, FENP group (test groups) showed significant (P<0.001) decrease in the tumor weight as compared to NC showing potential anti-tumor activities by all the test groups. FENP, showed significant (P<0.001) decrease in the tumor weight as compared to BNP and FE group showing more potent anti-tumor activity of FENP as compared to BNP and FE. When FE groups were compared with STD groups, no significant different was found amongst these groups showing equipotent anti-tumor activities of both the groups as that of STD group, no significant different was found.

3.2.8.3.6. Hematological Parameters

Thrombocytosis can be an early indicator of cancer. An increased risk of thrombosis as well as increased platelet activation has been observed in breast cancer. Results Figure 16 (a) and (b), showed that there was significant (P<0.001) decrease in the RBC, WBC and Hb count whereas significant increase in NC group as compared to PC group showing the severe alterations in hematological parameters due to induction of tumor in NC group. STD, BNP, FE, FENP group (test groups) showed significant (P<0.001) normalization in the hematological parameters as compared to NC showing potential protective activity of all the test groups against tumor induced hematological toxicities. FENP showed significant (P<0.001) increase in the RBC, WBC and HB count and significant (P<0.001) decrease in platelet count as compared to BNP and FE group showing more potent anti-tumor activity of FENP as compared to BNP and FE. When FE groups were compared with STD groups, no significant different was found.

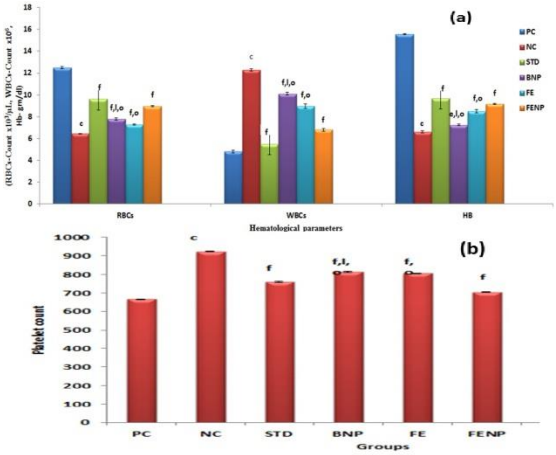

Figure 16: (*a) Effect on RBCs, WBCs, and Hb count, (b) Effect on Platelet Count*

The results are expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. NC compared to PC: a-P<0.05, b-P<0.01, c-P<0.001; all treatment groups compared to NC: d-P<0.05, e-P<0.01, f-P<0.001; FENP compared to STD: g-P<0.05, h-P<0.01, i-P<0.001; BNP and FE compared to FENP: j-P<0.05, k-P<0.01, l-P

3.2.8.4. Statistical analysis

The comparison between the groups was made by one way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test. In Vivo studies suggested that all the observed tissues of Rat treated with Standard drug, FE, FENP, and BNP did not revealed any lesion of pathological significance and metastasis of tumor. When compared with negative control group, the experimental group of animal of Standard drug, FE, FENP and BNP treated Female Rats revealed reduced size, distribution and severity of tumor

area. Further, increased necrosis at tumor site of treated Female Rat suggests mitigatory effect. It has been noted that experimental animal treated with nanoparticles revealed higher efficacy than extracts and blank nanoparticles.

4. CONCLUSION

The study proved that, the Brassica oleracea var. Italica Plenck. Floret extract was capable of producing silver nanoparticles using green synthesis technique in association with silver nitrate. These nanoparticles were characterized and evaluated for various tests. The results of the above tests were showing promising results. The study of green synthesis of silver nanoparticles of Broccoli could be an ecofriendly method for avoiding harmful effect in medical application of silver nanoparticles synthesized by physical, chemical, photochemical and radiation assisted process. It is also being expected that Green Silver Nanoparticles could be an alternative drug delivery system in treatment of Cancer considering harmful effects of other methods of treatment. The study proved the stated hypothesis that final formulation may show significant change in anticancer potential than individual. This study makes an attempt to overcome the limitations of conventional treatments of cancer with cost effective, ecofriendly, stable and safe targeted drug delivery as an alternative and / or complementary method of treatment.

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CONFLICT OF INTEREST

The authors declared that there are no conflicts of interest.

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