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An Eco-benign Synthesis of AgNps using Hydroalcoholic Extract of *Brassica oleracea var. italica* Plenck : Anticancer Response against Human Breast Cancer Cells MCF-7

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ABSTRACT

Breast Cancer is the second foremost reason of Cancer mortality in females globally. The conventional treatments available for Breast Cancer include surgery yet they are related to serious side effects that have moved the worldwide focus towards Complementary and Alternative Medicines (CAM). One of the emerging strategies has been the use of plant extracts for synthesizing metal nanoparticles (such as gold and silver) for anticancer applications. The objective of this study is to reflect the current availed study on green synthesis of silver nanoparticles (AgNps) with its future prospects to treat Breast Cancer. The development of eco-friendly and reliable techniques for silver nanoparticles synthesis is a vital initiative in the area of nanotechnology. *Brassica oleracea var. italica* Plenck Leaves Extract (LE) prepared by maceration process and silver nanoparticles of LE prepared by using biological reduction method. Female rats were divided into 5 groups, Group-I served as Positive Control and received normal saline. Group-II served Negative Control (Tumor Bearing) and was treated with single dose of MCF 7. Breast Cancer Cells (1.7 mg/pellet). Group-III served Standard Control (Tumor Bearing) treated with Paclitaxel 40 mg/Kg. Group-IV served test and treated with LE 400 mg/Kg, Group-V served test and treated with LENP 400 mg/Kg respectively about 21 days and on 21th day blood samples were collected for Hematological Parameters and Feed and Water Consumption, Body Weight Determination and Organ Weight were estimated. These discoveries infer that the synthesized Silver nanoparticles utilizing green nanotechnology could be a perfect methodology to battle malignant growth and irresistible ailments.

Keywords: Breast Cancer, Nanotechnology, Synthesis, , MCF-7, CAM

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1. INTRODUCTION

Breast Cancer is the second most leading cause of cancer death in women worldwide, and is predictable to surpass heart diseases in the next few years [1]. It accounts for around 7% of global Cancer burden and one-fifth of all the Cancers in India [2]. As indicated by the American Cancer Society, a gauge of 29% occurrences and 15% deaths due to Breast Cancer around the world (Siegel et al., 2014) has been predicted. 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 [3].

Herbal medicine that forms an integral part of CAM had reported to play an indicative part in the management of Breast Cancer [4]. Even though medicinal plants) and their bioactive have been reported to be highly potent anticancer agents, the widespread use of herbal bioactive has been restricted due to their hydrophobic nature that reduces their bioavailability and thusly diminishes their helpful adequacy [5]. This issue has been overcome with the advent of nanotechnology, which has had a significant effect on the advancement of Novel Drug Delivery Systems (NDDS) [6]. Lots of efforts have been undertaken to use Modern Nanotechnology to deliver herbal based drugs [7] for safer and more effective treatment of Breast Cancer. One of the emerging strategies has been the use of herbal extracts for synthesizing metal nanoparticles (such as gold and silver) for anticancer applications. Despite the fact that among the different biological methods for silver nanoparticle amalgamation, microorganism intervened combination isn't of industrial feasibility because of the prerequisites of exceedingly aseptic conditions and their support. Based on

the above background, the purpose of this study was to interrogate the capability of nanoparticles in delivery of herbals or their bioactive in Breast Cancer Cells. It involves synthesis, characterization and biological studies of nanoparticles conjugated with *Brassica oleracea var. italica* Plenck against Breast Cancer.

2. MATERIALS AND METHODS

2.1 Authentication of Plant

The plant was recognized and validated from Botanical Survey of India, Western Regional Center, Pune, Maharashtra, India.

2.2 Preparation of extract of *Brassica oleracea var. italica* Plenck leaves.

1000 gm of Leaves 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 is then allowed for evaporation of solvent. After evaporation, extract of *Brassica oleracea var. italica* Plenck leaves (LE) was weighed [8].

2.3 Biosynthesis of silver nanoparticles by using LE

Silver Nitrate concentrations ranging from 1 mM, 2 mM and 3 mM for silver nanoparticles synthesis were used and 1 ml, 1.5 ml and 2 ml. LE concentrations were used in (5:1) concentrations. Prepared solution of Extract was placed drop wise in $AgNO_3$ solution. Colour change was observed after time interval of 30 min, 60 min, 90 min, 120 mins, 150 min, 180 min, 12 h, 24 h and 48 h as the arrangement diverted into dark colored from yellow arrangement at room temperature proposing development of silver nanoparticles. [9].

2.4 Experiment design for Anticancer Activity

2.4.1 *Animals*

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.

All female rats were divided into 5 groups each group contain 6 animals.

Group-I served Positive Control and received normal saline.

Group-II served Negative Control (Tumor Bearing) was treated with single dose of MCF 7 breast cancer cells (1.7 mg/pellet).

Group-III served as Standard Control (Tumor Bearing) treated with Paclitaxel 40 mg/Kg.

Group-IV served as Test and treated with LE 400 mg/Kg.

Group-V served as Test and treated with LENP 400 mg/Kg.

2.4.2 Acute Toxicity Study in Wistar Albino Rat.

Acute toxicity on oral administration of all samples at different doses of 300, 2000 and 4000 mg/kg was carried out. Sign of morbidity and mortality in animals during the period of experiment for acute toxicity was observed.

2.4.3. Induction of Breast Cancer (Murine Tumour Model)

Breasts were inoculated with estradiol-17β (1.7 mg/pellet) before an infusion of 1×10^7 MCF 7 breast malignant growth cells subcutaneously into inguinal locale of mammary fat cushion. The tumor size watched and estimated in three measurements with calipers each 2 days beginning at day 7. After confirmation of tumour induction by histopathology treatment was started. Standard and Test samples were injected in to Animals according to schedule [10].

2.5 Evaluation Parameters for Anticancer Activity

2.5.1 Feed and Water consumption

The measure of feed and water devoured for 24 h was estimated week by week from the amount of feed and water provided and the amount remaining after 24 h for till the end of the experiment.

2.5.2 Body Weight Determination

Body Weight of individual animal was noted week after week till end of the investigation.

2.5.3 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 also, utilized for the assessment of red blood cell count (RBC), Hemoglobin (Hb) content and white blood cell count (WBC). Examination was made among all groups. Blood was immediately transferred into EDTA bulbs for hematological parameters examination.

2.5.4 Organ Weight

The organs under examination were extracted from the animal and weighed. The weights of the organs, such as, Lung, Liver, Kidney, Heart, Brain and Spleen were recorded and contemplated for any strange put on or loss of weight. This gives a primer affirmation with respect to the adverse effects (assuming any) of the medication under test. The weights of the organs expressed as relative loads as g/100 g b.w. and determined by following equation:

$$\text{Relative Organ Weight} = \frac{\text{Absolute Organ Weight(g)}}{\text{Body Weight of rat on sacrifice day(g)}} \times 100$$

2.5.5 Statistical analysis

All analyses were carried out in triplicate, freely, and the outcomes were communicated as the mean ± SD. Measurable examinations of the information were performed utilizing the Graph Pad Prism8 programming, variant 8.01. Results were communicated as mean ± SD of the demonstrated number of autonomous trials. Investigation of difference (ANOVA) was utilized to think about the mean estimation of information and $P<0.05$ were treated as significant.

3. RESULTS AND DISCUSSION

3.1 Identification and authentication:

The plant was identified and authenticated as *Brassica oleracea var. italica* Plenck (Family: Brassicaceae) from Botanical Survey of India, Western Regional Centre, Pune (BSI), Maharashtra, India. **(Voucher specimen No. RBC-3, BSI /WRC /IDEN.CER /2016/667).**

3.2 Visual Observation

Visual perception of colour change from yellow to dark brown after addition of extract to $AgNO_3$ was observed demonstrating the biosynthesis of AgNps by diminishing Ag^+ particles with the concentrate as topping operators as shown in Figure 1.

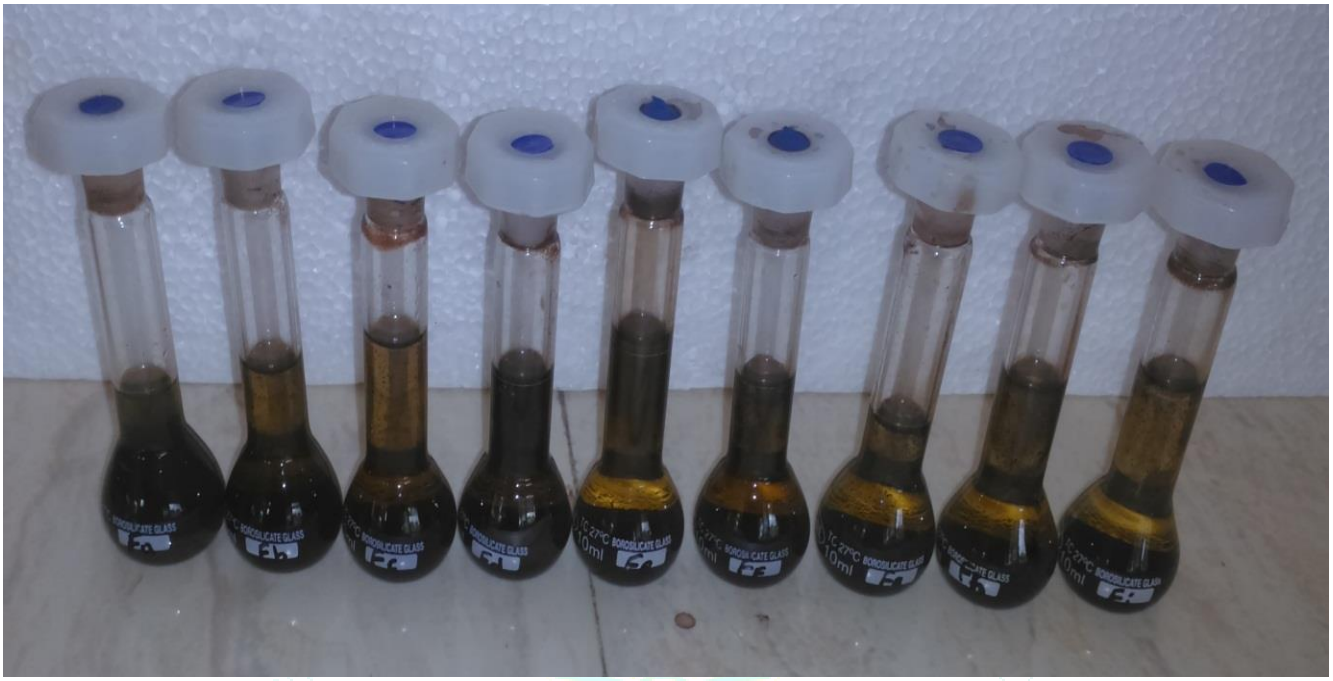

Figure 1: Confirmation of Formation of *Brassica oleracea var. italica* Plenck Leaves Extract Loaded AgNps.

3.3 Acute Toxicity

Test samples were subjected to acute toxic effect as per the guideline 423 of OECD, wherein 4000 mg/kg was used as the limit test dose. Toxic symptom or mortality related to treatment were not observed after test sample administration by oral route at 300, 2000 and 4000 mg/kg dose.

3.4 Murine Tumour Model

Murine tumor model was chosen to decide the anticancer potential of extracts and formulations. After the cell line treatment Confirmation of tumour formation was done by Histopathology. Studies noted multifocal mild neutrophilic/lymphocytic infiltration and multifocal mild neovasularization 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 without demonstration of gland formation was noted (variations in nuclear size and staining), 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 neovasularization (3+) indicated Low grade Solid Mammary Adenocarcinoma.

Diffusely severe tract of neoplastic cells spread across complete subcutaneous area with diffuse moderate stroma along with fibrous tissue is observed in negative control group experimental animal. Moderate cellular pleomorphism with round to oval nucleus and pink cytoplasm, moderate anaplasia and increased mitotic is also noted. Focal mild necrosis and diffuse moderate inflammation at tumor site with neovasularization is seen in positive control animal.

When compared with experimental animal of negative control group, experimental animal of Standard drug, LE and LENP 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.

3.5 Effect on Treated Animals

3.5.1 Feed and Water Consumption

The amount of feed and water consumed for 24 hours was estimated week by week from the amount of feed and water provided and the amount remaining after 24 h till the end of experiment.

After administration of investigating compounds, the study period significantly affected the feed intake. After Injection of Cell line Negative Control Group had the lowest feed intake as compare to the Normal Control Group. Followed by Test Group (LE, LENP) the food intake of rats significantly increases as compared to Negative Control Group as demonstrated in Table 1.

Table 1: Feed Consumption

Group	Before Cell line Injection	At the time of Cell line Injection	After Treatment
PC	47.5 ± 4.58	48.6 ± 6.45	47.3 ± 4.58**
NC	50.5 ± 7.54	27.3 ± 4.78	25.3 ± 7.59***
STD	55 ± 4.56	36.5 ± 5.48	45.3 ± 6.45***
LE	53.6 ± 4.89	31 ± 6.89	38 ± 4.96**
LENP	46 ± 4.99	34 ± 6.41	41.2 ± 3.96**

All the values are expressed as mean ± SD (n=6); Vs Normal Control; *p<0.05, **p<0.01, ***p<0.001 Vs Negative Control. Whereas, PC: Positive Control, NC: Negative Control, STD: Standard Control LE: Leaves Extract (Test Sample), LENP: Leaves Extract mediated AgNps (Test Sample).

3.5.2 Water Consumption

After administration of cell line, the water intake of negative control group significantly decreases as compared to Normal Control Group. However, in test and standard compound treated group the water intake capacity of rats significantly increase compared to Negative Control Group as shown in Table 2.

Table 2: Water Consumption

Group	Before Cell line Injection	At the time of Cell line Injection	After Treatment
PC	189 ± 1.23**	189.5 ± 2.12	185.14 ± 2.89**
NC	187 ± 1.56***	124.5 ± 2.65	115 ± 2.98***
STD	190 ± 2.01*	147 ± 3.14	168 ± 3.44**
LE	185 ± 2.66**	134.5 ± 2.44	144.6 ± 3.11**
LENP	184 ± 3.66***	128.5 ± 1.89	160 ± 2.87***

All the values indicated as mean ± SD (n=6); Vs Normal Control; *p<0.05, **p<0.01, ***p<0.001 Vs Negative Control. Whereas, PC: Positive Control, NC: Negative Control, STD: Standard Control LE: Leaves Extract (Test Sample), LENP: Leaves Extract mediated AgNps (Test Sample).

3.5.3 Body Weight Determination

The body weight is a parameter considered as an indicator of symptoms in different organs. Table 3 demonstrates the weights of rats after administration of investigating compound. The data shows convincing differences between Positive Control and Negative Control Group. The Negative Control Group had significantly increased body weight in comparison to Positive Control Group. However, towards the end of the experiment, slight differences in weights were seen in all the test and standard groups contrasted with control as shown in Table 3.

Table 3: Body Weight Determination

Group	Before Cell line Injection	At the time of Cell line Injection	After Treatment
PC	171.25 ± 2.05**	180.75 ± 1.54	182 ± 5.65***
NC	181.25 ± 3.60***	196.75 ± 2.69	200 ± 1.58*
STD	138.5 ± 4.02*	154.25 ± 7.21	170.2 ± 3.62**
LE	151.25 ± 4.54**	172 ± 3.65	176.75 ± 1.32**
LENP	139.75 ± 2.44***	157 ± 2.78	162 ± 2.78***

All the values are expressed as mean ± SD (n=6); Vs Normal Control; *p<0.05, **p<0.01, ***p<0.001 Vs Negative Control. Whereas, PC: Positive Control, NC: Negative Control, STD: Standard Control LE: Leaves Extract (Test Sample), LENP: Leaves Extract mediated AgNps (Test Sample).

3.5.4 Organ Weight

Organ Weight can be the most delicate marker to know the impact of an experimental task and made a decision by significant differences in Organ Weight among treated and untreated animals Liver, Kidney Lungs, Heart, Spleen and Brain have been used to evaluate safety of test drugs as shown in Table 4.

Table 4: Organ Weight

AVG.WT(gm) /GROUP	LIVER	KIDNEY each	LUNGS	HEART	SPLEEN	BRAIN
PC	5 ± 0.11*	0.5 ± 0.01	2 ± 01	0.5 ± 0.01	1.5 ± 0.16	1.5 ± 0.12
NC	13.5 ± 2.55*	0.5 ± 0.02	2 ± 04	0.5 ±0.03	1.4 ± 0.23	1.4 ± 0.54
STD	6.2 ± 1.22**	0.5 ± 0.01	2.1 ± 03	0.5 ± 0.05	1.5 ± 0.24	1.5 ± 0.42
LE	7.6 ± 3.11*	0.5 ± 0.03	2.4 ± 0.5	0.5 ± 0.04	1.4 ± 0.54	1.5 ±0.54
LENP	6.8 ± 1.66 ± 2.1*	0.5 ± 0.11	2.3 ±0.7	0.5 ± 0.02	1.5 ± 0.78	1.4 ± 0.78

All the values are revealed as mean ± SD (n=6); Vs Normal control; *p<0.05, **p<0.01, ***p<0.001 Vs Negative control. Whereas, PC: Positive Control, NC: Negative Control, STD: Standard Control LE: Leaves Extract (Test Sample), LENP: Leaves Extract mediated AgNps (Test Sample).

3.5.5 Hematological Parameters

Furthermore, hematological indices in animals are critical to decide the toxicity risk since the variation in the blood system has greater prescient value in regard of human toxicity. Statistics in Table 5 show the concentration of RBC, Hb content, WBC, MCV, PCV, MCH, platelets count, MCHC, lymphocytes and neutrophils of all groups. These hematological parameters were significantly lower in Negative Control Group. While in test compounds and standard drug significantly increases as compared to Negative Control Group as demonstrated in Table 5.

Table 5: Hematological Parameters

Parameter	Positive Control	Negative Control	Std	LE	LENP
WBC ($X10^3/\mu L$)	4.87 ± 1.23	17.2 ± 1.01**	12.1 ± 2.31**	13.0 ± 1.11*	9.1 ± 0.66**
RBC ($X10^6/\mu L$)	12.3 ± 1.59	6.2 ± 2.01*	8.55 ± 1.26*	7.18 ± 2.12**	8.91 ± 2.11**
Hgb (g/dL)	15.7 ± 1.96	6.6 ± 1.23***	7.9 ± 2.55*	8.5 ± 1.09***	8.4 ± 1.22*
MCV (fl)	56.2 ± 2.14	41.3 ± 4.56**	50.5 ± 1.45**	53.6 ± 2.36**	53.8 ± 2.11**
MCH (pg)	20.5 ± 5.12	11.2 ± 2.45*	15.6 ± 2.11*	14.8 ± 1.12**	13.5 ± 2.32*
MCHC (g/dL)	35.5 ± 3.36	33.3 ± 3.21**	35.4 ± 1.44**	35.2 ± 1.89*	34.4 ± 1.96***
PLT ($X10^3/\mu L$)	663 ± 4.52	917 ± 2.15**	758 ± 5.55*	805 ± 4.12**	760 ± 4.55*
Neutrophil (%)	24 ± 2.12	41 ± 1.23*	33 ± 2.55**	36 ± 2.16*	31 ± 2.11**
Lymphocyte (%)	73 ± 1.25	109 ± 1.47***	80 ± 3.12**	89 ± 2.10**	82 ± 1.22**
Monocyte (%)	0.2 ± 1.35	3 ± 1.13*	1 ± 0.01*	3 ± 1.11**	1 ± 0.25**
Eosinophil (%)	0.4 ± 0.12	1 ± 1.54**	1 ± 0.19*	2 ± 0.63*	2 ± 0.12*
Basophil (%)	0 ± 0.00	0 ± 0.00ns	0 ± 0.00	0 ± 0.00	0 ± 0.00

All the values are expressed as mean ± SD (n=6); Vs Normal Control; *p<0.05, **p<0.01, ***p<0.001 Vs Negative Control. Whereas, PC: Positive Control, NC: Negative Control, STD: Standard Control LE: Leaves Extract (Test Sample), LENP: Leaves Extract mediated AgNps (Test Sample).

4. CONCLUSION

Silver Nanoparticles (AgNps) were prepared by biological reduction method using *Brassica oleracea var. italica* Plenck Leaves extract. Synthesis of AgNps by biological reduction method is a sort of base up methodology wherein reduction is the fundamental reaction which was taken place. The decrease of silver particles prompts the development of stable silver nanoparticles. This work aimed to synthesis of a novel drug delivery system with herbal drug *Brassica oleracea var. italica* Plenck in order to reduce possible side effects of conventional treatment. *In-Vivo* studies suggest that *Brassica oleracea var. italica* Plenck Leaves extract mediated Silver Nanoparticles exhibit anticancer activity. These discoveries infer that the synthesized nanoparticles utilizing green nanotechnology could be a perfect procedure to battle malignant growth and irresistible illnesses.

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