# PRE-PUPAL STAGES OF B.S.F. *HERMETIA ILLUCENS* (L.) FOR TREATING THE CELLS OF CANCER OF THE COLON IN NORWEGIAN RAT, *RATTUS NORVEGICUS* (L)

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**Abstract**
*Drug resistance capabilities and scarcity of effective drug are supposed to be the obstacles in the method of treatment for cancer of colon. The methanol solution (100 PPM) maceratives of pre-pupal stages of B.S.F. Hermetia illucens (L.) and zingiberene (Terpene of "Monocyclic-Sesquiterpene" class), significant constituent of Zingiber officinale (L.) were assessed for treating the cells of cancer of the colon belong to Norwegian rat, Rattus norvegicus (L). The cell counting assay was utilized for assessment of rate of proliferation of the cells of colon cancer. Through the use of transmission electron microscopy, the detection of autophagy was carried out. The varied concentrations of monocyclic-sesquiterpene, zingiberene and the methanol maceratives of pre-pupal stages of B.S.F. Hermetia illucens (L.) used for treating the "Transfected cells" derived from rat colon include: 000.00 microliters; 100.00 microliters, 150.00 microliters and 200.00 microliters. The fluorescent microscopy was utilized for monitoring. The method of flow cytometry was used for analysis of cell cycle. Expression of the protein was carried out through the immunoblotting. The zingiberene (Terpene of "Monocyclic-Sesquiterpene" class), significant constituent of Zingiber officinale (L.) and the methanol maceratives of pre-pupal stages of B.S.F. Hermetia illucens (L.) were found considerably inhibiting the proliferation of cancer cells of colon from Norwegian rat, Rattus norvegicus (L). Induction of autophagy through the zingiberene and the methanol maceratives of pre-pupal stages of B.S.F. Hermetia illucens (L).*

## Introduction

Most of the plant derived and animal derived compounds and antimicrobial proteins are working as anticancer agents because of their potent activity. They are also known for lowering down the side effects[1]. Taxol like herbal products are utilized for the management to control the cancer[2]. There is a wide array spectrum of metabolites produced by the plants, animals and microbials. They can be classified into various categories on the basis of chemical structure and physical characters[3]. The group of sesquiterpenes constitutes the largest assortment of bio-molecules. They are prevalently found in most of the plants[4]. The sesquiterpenes of most of the plants have exhibited the capacity to inhibit the proliferation of the cells of cancer, such as lung cancer, breast cancer and gastric cancer to name a few[5]. The immense potential of sesquiterpene bio-compounds made man to reach clinical trials[6]. The zingiberene is a sesquiterpene (monocyclic) belongs to most of the plants. The major source of zingiberene is ginger, *Zingiber officinale* (L)[7]. The ginger, *Zingiber officinale* (L) has been reported for capabilities of inhibition of the growth of different types of cells of cancer[8].

The abnormal growth of cells appears to the distinguishing character of cancerous tissue. The microenvironment for cancer tissue is multifaceted and complex. The microenvironment for cancer tissue is composed of multiple cell types; extracellular matrix and associated stromal cells; and endothelial cells and associated structures (blood vessels) and the local milieu representing a complex mixture of soluble factors. The multiple cell types in cancer tissue include: neoplastic, normal, and reactive. The complex mixture of soluble factors in cancer tissue are derived from both the neoplastic cells and normal cell types. It is important to note that, the microenvironment of any tissue exert influence the quality of the growth. If this microenvironment of tissue is with carcinogens, it leads to exert an emergent tumour. The cancer tissue deserves the ability to modify its tissue microenvironment. Most of the cancer tissues have an inflammatory cell infiltrate. This cell infiltrate ranges from microscopic or subtle inflammation (a few infiltrating cells of specific subtype) to gross inflammation. The gross inflammation of cell infiltrate is recognizable using standard histologic stains. The gross inflammation of cell infiltrate[9]. In a real sense, cancer tissue is formed through the group of diseased cells. The abnormal cell growth in cancer tissue is with the potential to invade or spread to other parts of the body. Formation of a lump; bleeding with abnormal rate; cough for prolonged duration; loss of body weight (without understanding the cause) and changes in the movements of bowel are some of the signs and symptoms of cancer. The factors responsible for the initiation and growth of cancer are recognized as, the "Carcinogens". The carcinogens are with ability of damaging the genome and / or disrupt the cellular metabolism. Miyata, *et al* (2001)[10],

reported the immunosuppressive activity of the 7,12- Dimethylbenz [a] anthracene (DMBA) is an immunosuppressor and recommended as a powerful organ-specific to be used for studies in laboratory as carcinogen. Therefore, 7,12-Dimethylbenz [a] anthracene (DMBA) is widely used in number of attempts of cancer researches and laboratories for the studies of the cancer. The 7,12-Dimethylbenz [a] anthracene (DMBA) is working as a tumour initiator. Sung, *et al* (2005)[11] reported the tumour promotion through the treatment of 12-O-tetradecanoylphorbol-13- acetate (TPA). The 12-O-tetradecanoylphorbol-13- acetate (TPA) allows for a greatly accelerated rate of tumour growth, making many cancer studies possible.

**Material And Methods**

The experimentation on the use of methanol maceratives of pre-pupal stages of B.S.F. *Hermetia illucens* (L.) for treating the cells of cancer of the colon in Norwegian rat, *Rattus norvegicus* (L) was divided into the attempts like: Procurement and culture of colon cancer cells; Treatment with zingiberene and methanol maceratives of pre-pupal stages of B.S.F.; Assessment of cell viability and colony formation; Transmission Electron Microscopy (TEM) for the Detection of Autophagy; Transfection assay for demonstration of autophagy-induction; Xenograft; Western blotting and Statistical analysis.

(1). Procurement and Culture of Colon Cancer Cells Derived from Norwegian rat, *Rattus norvegicus* (L): "Colo-786", the colon cancer cell lines were supplied by the National Centre for Cell Science, Savitribai Phule Pune University. The serum-supplemented medium consisted of RPMI-1640 growth medium, serum-free medium, Dulbecco's modified Eagle's medium and Ham's nutrient mixture. Colon Cancer Cells were sub-cultured in Serum Supplemented medium. The Cells at the exponential growth phase were washed with Phospahte Buffer Saline and digested with trypsin. They were allowed for the formation of cancer spheres. The method described by Paraskeva, *et al*. (1984)[38] was used to maintain the culture of colon cancer cells in laboratory.

(2). Treatment with zingiberene and methanol maceratives of pre-pupal stages of Black Soldier Fly (BSF): The cancer spheres yielded in the attempt of procurement and culture of colon cancer cells derived from Norwegian rat, *Rattus norvegicus* (L) were used for seeding in separate well-plates. The well-plated were processed for incubation for twenty-four hours separately with Zingiberene and methanol solution of prepupal stages of black soldier fly. The cell-aliquots were then removed and processed for counting in triplicate set through the method of Trypan blue staining. The effect of Zingiberene and methanol solution of prepupal stages of BSF on the formation of colonies of "Colon cancer cell lines" was investigated as described by Bruce, *et al*. (1966)[39].

(3). Assessment of cell viability and colony formation: For the assessment of cell viability and colony formation, the method of "cell counting assay" was utilized. Normal healthy cells of colon of Norwegian rat, *Rattus norvegicus* (L) (CCD-1963Co and the colon cancer cells (Colo786) were utilized for this attempt. The cells/well (5×104) were seeded in twelve well plates and allowed for incubation for twenty-four hours with different concentrations of zingiberene and methanol solution of prepupal stages of black soldier fly. The aliquots of cells were then removed and counted in triplicate following Trypan blue stain. The cell-aliquots were then removed and processed for counting in triplicate set through the method of Trypan blue staining. The effect of Zingiberene and methanol solution of prepupal stages of BSF on the formation of colonies of "Colon cancer cell lines" was investigated as described by Bruce, *et al.* (1966)[39]. The effect of zingiberene and methanol solution of prepupal stages of BSF on the formation of colonies of "Colon cancer cell lines" was studied.

(4). Through the method for the transmission electron microscopy (TEM) for the detection of autophagy in cancer cells explained by Hai Chen, *et al*. (2019)[40], the present attempt was carried out. Transmission Electron Microscopy (TEM) for the Detection of Autophagy in Cancer Cells: Transmission electron microscopy available at national centre for cell science (NCCS, Pune) was utilized for the detection of autophagy through the assessment of the induction of autophagy in cancer cells. The cells of all the groups (untreated control; solvent treated control; zingiberene treated and the cells treated with methanol solution of prepupal stages of BSF) were processed for fixing them in four percent glutaraldehyde solution; solution of sodium cacodylate (0.05M); postfixing in 1.5 percent osmium tetroxide (OsO4). The cells of all the groups (untreated control; solvent treated control; zingiberene treated and the cells treated with methanol solution of prepupal stages of BSF) were then processed for dehydration through the use of various grades of alcohol. The cells of all the groups (untreated control; solvent treated control; zingiberene treated and the cells treated with methanol solution of prepupal stages of BSF) were then, prepared for flat embedding in Epon 812. The cells of all the groups (untreated control; solvent treated control; zingiberene treated and the cells

treated with methanol solution of prepupal stages of BSF) were finally processed for observation through the use of Zeiss CEM 902 electron microscope at national centre for cell science (NCCS, Pune).

(5). Transfection assay for demonstration of autophagy-induction: Through the method for the transfection assay for the demonstration of autophagy-induction in cancer cells explained by Xue Han, *et al*. (2018)[41] and Hai Chen, *et al*. (2019)[40], the present attempt was carried out. The "Colo-786", the colon cancer cells were allowed for their growth up to eighty percent confluence. Through the guidelines of manufacturer, "Colo-786", the colon cancer cells were transfected with GFP-LC3 plasmids (through the use of Invirogen: Lipofectamine 2000 as per the guidelines of manufacturer). The GFP-LC3 plasmids are mammalian expression vector. The GFP-LC3 plasmids are containing the gene: LC3B (human or murine) that are fused at its 5' end to the "Green Fluorescent Protein" (GFP) gene. The GFP-LC3 plasmids are selectable in bacterial cells and mammalian cells through the use of "Zeocin" (Zeocin is antibiotic of glycopeptide category and one of the phleomycins derived from *Streptomyces verticillus* L.). The expression of the transfected with GFP-LC3 plasmid fusion gene is going help to visualize the formation of autophagosome in a real time in the live cells. During the process of formation of autophagosome, GFP-LC3 plasmid is carried to recruit to the membrane of autophagosome. Through high resolution fluorescence microscopy, here, it can be imaged as cytoplasmic puncta. The percentage of cells positive with GFP-LC3 can be determined. This is indication of formation of autophagosome. The treatment of Zingiberene and methanol maceratives of pre-pupal stages of Black Soldier Fly (BSF) (concentrations: 0.0; 10.0, 20.0 and 40 micromoles) separately to the transfected cells was then carried out. The duration of treatment of Zingiberene and methanol maceratives of pre-pupal stages of Black Soldier Fly (BSF) was twenty-fours. The fluorescent microscopy was used to monitor.

(6). Xenograft (In vivo attempt): The method explained by Hai Chen, *et al*. (2019)[40] was used to carry out the Xenograft (In vivo attempt). The xenograft studies deal with procurement of a tissue (or organ) derived from a species of completely different from the recipient animal of experimentation. The xenograft studies are powerful tools of research, especially in oncology. Healthy Norwegian rat, *Rattus norvegicus* (L) with average weight of 25 ± 2.0 g were obtained from the Department of Zoology, Savitribai Phule Pune University, Pune. At random, Norwegian rats (*Rattus norvegicus* L.) were divided into two control groups (untreated control; solvent treated control); three groups treated with zingiberene dissolved in 0.1 percent methanol (at ten, twenty and forty milligrams per Kg) and three groups treated with BSF powder dissolved in 0.1 percent methanol (at ten, twenty and forty milligrams per Kg). Total eight groups of the experimental animals (Norwegian rats, *Rattus norvegicus* L.) were maintained in laboratory. Each group was with ten individuals. The colon cancer cells were subcutaneously injected into the left flank of each individual experimental animal in each group. The experimental animals (Norwegian rats, *Rattus norvegicus* L.) were monitored and as the growth of the tumour was apparent, they (Zingiberene treated groups and BSF treated groups) received Zingiberene and BSF as per the schedule. The animals (Norwegian rats, *Rattus norvegicus* L.) of untreated control received no treatment. The animals (Norwegian rats, *Rattus norvegicus* L.) of solvent treated group received 0.1 percent methanol in normal saline thrice weekly. At the end of the fifth week, all the experimental animals (Norwegian rats, *Rattus norvegicus* L.) were euthanized for harvesting the tumours. The harvested tumours were utilized for further experimental analysis.

(7). Western Blot: The western blot (also called as Western Blotting) is dealing with detection of specific proteins from assay sample obtained from homogenate of the tissue. The method explained by Hai Chen, *et al*. (2019)[40] was used to carry out the western blotting in present attempt. Through the use of ice-cold phosphate buffer saline (PBS), the colon cancer cells (Colo786) were washed. The PBS washed colon cancer cells (Colo786) were then suspended in a lysis-buffer at 4°C and then shifted to 95°C. Through the use of Barford assay method, the content of proteins from each assay sample was examined. About, forty micrograms of protein were used to load from each assay sample. Before being shifted to polyvinylidenefluoride membrane, the protein contents from each assay sample were separated by the "Sodium Dodecyl Sulfate–Polyacryl-Amide Gel Electrophoresis (SDS-PAGE). The polyvinylidenefluoride membranes were then treated with "tris-buffered-saline" (TBS). At 4°C, the polyvinylidenefluoride membranes treated with "tris-buffered-saline" (TBS) were exposed to primary-antibodies. The appropriate secondary-antibodies were used for treating the cells. Finally, the expected proteins were used to visualize through enhanced chemiluminescence reagent. Thus, the "Western-Blotting" is helping for the detection of protein

samples immobilized on a nitrocellulose or polyvinylidene fluoride (PVDF) membranes.

(8). Statistical analysis:

Each and every attempt in experimentation was performed in triplicate. The data were presented as mean, standard deviation. SPSS version seventeen was used to carry out the statistical analysis. The results were presented through Student's t-test and one-way ANOVA. "P" less than 0.05 was considered statistically significant. The full form of "SPSS" is Statistical Package for the Social Sciences. It is software for statistical analysis developed by IBM[42]. It is used for data management, analytics, analysis of multivariate type, etc.

**Results And Discussion**

The results on assessment of the methanol maceratives of pre-pupal Stages of B.S.F. *Hermetia illucens* (L.) for treating the cells of cancer of the colon in Norwegian rat, *Rattus norvegicus* (L) are summarized in Fig. 1; Fig.2; Fig.3 and Fig.4 and explained away through the points including: Cell Viability; Autophagy; Xenograft and Western Blotting.

1.Cell Viability:

The growth inhibitory effects of Zingiberene and BSF powder were assessed on the cancer cell line. The treatment with zingiberene and BSF powder caused dose-dependent inhibition of the growth in cancer cells. The measure of potential (or potency) of any substance responsible for fifty percent inhibition, in vitro, for the expected biochemical process is termed as, "Half Inhibitory Concentration" ($IC_{50}$). It is a quantitative measure indicating amount of a specific substance of inhibition. The "Half Inhibitory Concentration" ($IC_{50}$) for zingiberene and BSF powder against the cancer cells in present attempt were twenty and sixty-five microliters respectively (Fig.1). The treatment of zingiberene and BSF powder, additionally also induced some morphological changes. The cells derived from the cancer tissue are with significant genomic signature of primary tumour, from which cells were sourced[43,44]. The results obtained through the use of such cell-lines is significantly predictive of subsequent clinical outcomes[44]. In each and every attempt on improvement in the efficacy of cancer preventing substances and going to enter in clinical trial, it is necessary for determination of the efficacy of such substances in vitro as precisely as possible. With reference to determination of the efficacy of anticancer substances, process starts with proper method of assay in understanding, selection of the method, and execution of the clinical trials. The cell viability assay method is useful for determination of the quantitative number of cells remained live after treatment with the anticancer substances. The assay through the formation of a colony by the cancer cells is directly illuminating the abilities of anticancer substances to inhibit proliferation of tumour cells. The assay method on cytotoxicity is demonstratable for the visualization of living and dead cells after the use of anticancer substances. The assay method on cellular apoptosis is allowing researchers for quantification of the number of apoptotic-cells after the treatment. The assay method on arresting the cell cycle is allowing to know the number of apoptotic-cells deceased at specific phase within the cell-cycle. Each and every assay method deserve several strengths and limitations. No single method is going to prove cent percent efficacy. It is significant for the purpose to strategize and to follow multiple method system to gain the reliable results.

The morphological changes in colon cancer cells through the treatment of zingiberene and BSF powder include: shrinkage and membrane blebbing of the cancer cells (Fig.2). The treatment with Zingiberene and BSF powder suppresses the growth of colon cancer cells. Both the treatments in present attempt caused dose-dependent inhibition on the growth colon cancer cells. However, the toxic effects of zingiberene and BSF powder on the non-cancer were significantly negligible. The influence of treatment of Zingiberene and BSF powder were also studied on the formation of a colony among the colon cancer cells. It exhibited to exert a dosage dependent inhibitory influence on the colony formation among the colon cancer cells in present attempt.

2.Autophagy:

During the research work on the discovery of lysosome, Christian René Marie Joseph, Viscount de Duve (Birth: 2 October, 1917 – Death: 4 May, 2013)[45] very firstly coined the term, "Autophagy". This term, "Autophagy" coined by Christian René Marie Joseph, Viscount de Duve was for the purpose to elaborate the significance of lysosomes in cell metabolism. This instance led to Christian René Marie Joseph, Viscount de Duve being awarded the "Physiology or Medicine Nobel Prize in the year: 1974. The term, "Autophagy" was adopted for the purpose to distinguish the process of degradation of components of the cell itself from the process of taking and degradation of the substances of outside of the cell (extracellular substances). The process of taking and degradation of the substances of outside of the cell (extracellular substances) is termed as, heterophagy. The attempts of the studies on autophagy until the year: 1990 were largely mostly observational or based on morphological

observation. In the year: 1993 Yoshinori Oshumi (Birth: 9 February, 1945)[46] explained a screen on the basis of genetics in yeasts in the process of undergoing deprivation of nitrogen. This attempt made led Yoshinori Oshumi to the process of isolation of mutants with autophagy-defective. Now a days, the autophagy-defective mutants are recognized as, "ATG" (AuTophaGy-related) genes. The results on screen identification of mutants that fell into the fifteen groups of complementation implying that, at least fifteen genes were involved in the process of regulation of autophagy in the yeasts undergoing deprivation of the nutrients. Further research attempts elaborated forty-one genes concerned with: "Yeast ATG – Genes". Not all, but most of the genes concerned with: "Yeast ATG – Genes" many have orthologues in human beings. Surprisingly enough, the attempts on the identification of the genetic foundation of autophagy led to an explosion in its research and Yoshinori Oshumi was awarded the Nobel Prize in Physiology or Medicine (Year: 2016)[47].

In its simplest language, the digestion of parts of cell by itself through the lysosomes is called as autophagy. The autophagy is used to disassemble the unwanted or unnecessary or dysfunctional, long-lived proteins and damaged or older organelles for the purpose to recycle biomolecules. The cells of cancer cell deserve capability to hijack the autophagy mechanism for the purpose to survive under stress caused through problematic metabolism. During chemotherapy, autophagy may also be helping for cancer cells for the purpose to develop resistance. The present attempt is reporting triggered development of autophagic vesicles in cancer cells through treating the methanol solution of Zingiberene and BSF. The triggered development of the vesicles of autophagy was observed in the zingiberene and BSF powder treated colon cancer cells in the present attempt on analysis (through the use of electron microscopy at National Centre for Cell Science) of the influence of the zingiberene and BSF powder treatment.

The biological technique on fluorescent microscopy is utilized to obtain the image-specific features of specimens of microscopic size. The biological technique on fluorescent microscopy is also utilized to visual enhancement of three-dimensional features at microscopic scales. Microtubule-associated protein "1A/1B-light chain-3" (LC3) is a soluble protein. It is with a molecular mass of approximately 17 kDa. It is distributed in constantly encountered manner in the tissues of animals of Class- Mammalia. It is also distributed in constantly encountered manner in cultured cells. In the process of autophagy, the autophagosomes use to engulf cytoplasmic components, which include: proteins of cytosolic nature and the cell organelles. Utilization of the biological technique on fluorescent microscopy in present attempt showed that, both the treatments (zingiberene and BSF powder) enhanced expression of the microtubule-associated protein "1A/1B-light chain-3" (LC3). Analysis through the "Western Blotting" further confirmed the zingiberene and BSF powder induced autophagy in the colon cancer cells. The expression of the LC3-II expression was found increased and the expression of p62 was found decreased considerably through the both the (zingiberene and BSF powder) treatments. However, the LC3-I-expression exhibited no remarkable change. Both, zingiberene and BSF powder caused deactivation through dosage-dependency in colon cancer cells.

The "Protein kinase B" (PKB, or Akt) serve to play a crucial role in cellular metabolism, cellular growth, cellular proliferation, and cellular survival. The activation of "Protein kinase B" (PKB, or Akt) is controlled through a multi-step process involving "Phosphoinositide-3-kinase (PI3K)". The present attempt examined the influence of zingiberene and BSF powder on the signalling pathway of "PI3K/AKT" in colon cancer cells. This attempt was revealed that, the treatment of zingiberene and BSF powder exerted to cause decline in "Phosphorylation of mTOR, PI3K and AKT" with concentration dependency. There was no significant influence observed on the "Total mTOR, PI3K and AKT" – expression.

3. Xenograft:

Zingiberene is well recognized for its anticancer activity. Therefore, it is selected for comparison with the BSF powder in present attempt. In the present attempt, the effects of Zingiberene and BSF powder were investigated on the xenografted tumour growth in vivo. The results showed that the administration of Zingiberene and BSF at 0, 10, 20 and 40 mg/kg caused significant decrease in the tumour volume and weight of the xenografted tumours. Decrease in the volume of tumour in present attempt is the prime indication of the in vivo anticancer activity of BSF powder. Both, zingiberene and BSF powder inhibit the growth of the colon cancer cells by inducing autophagy. The present attempt is reporting anticancer activity of BSF powder. Hence, BSF is going to prove beneficial in treating colon cancer.

The colon cancer is ranking as the fourth most significant and prevalent type of cancer. The colon cancer exerts significant morbidity and mortality across the world[48]. The outcomes of clinical trials with the strategies of current treatments appears to be far from descent. The strategies of current clinical treatments on colon cancer are exhibiting

either the side effects or nonsignificant efficacy of the compounds used in treatments[35]. Therefore, it is prime concern for the science to identify the novel biomolecules working as efficient anticancer agent and subsequently to develop the regimes of treatments of the highest efficiency. The products of nature through plants and animals deserve impressive potential working as medicines to alleviate the disorders in human body. There are many products of nature through the plants and animals already being used as medicines among the human societies in the world[49]. The present attempt tried its best to analyse the potential of zingiberene and BSF powder working as anticancer agents. Both of them (zingiberene and BSF powder) exerted growth inhibition for colon cancer cell line. The present attempt is reporting zero toxic influence of both, zingiberene and BSF powder in colon cancer affected in Norwegian rat, *Rattus norvegicus* (L). Further, the present attempt confirmed the morphological changes (alteration in the morphology) and formation of colony by the colon cancer cell line. The previous attempts by other researchers have also proved the inhibition of growth of cancer cells by zingiberene, the significant constituents of *Zingiber officinale* (L)[50]. The process of autophagy is a vital process. It is helping in the removal of cellular products of dysfunctional categories. It is also expert in the process of elimination of the cells of abnormal categories from the body[51]. There are several sesquiterpenes, including "Zerumbone" exhibiting potential in the process of inducing autophagy. The "Zerumbone" is a natural sesquiterpene. It is reported to induce autophagy in the cells of cancer of human prostate gland. According to Chan, *et al*., (2015)[52], through the oxidative stress, the "Zerumbone" is inducing the autophagy in the cells of cancer of human prostate gland. "Bigelovin", a sesquiterpene lactone for inducing the autophagy in liver cells cancer[53]. Chen, *et al*, (2019)[54] considered "The mTOR/PI3K/ AKT signal transduction pathway" in regulation of the proliferation and tumorigenesis of cancers of several types. The present attempt reporting, the process to block this "mTOR/PI3K/ AKT signal transduction pathway" through inhibition of the phosphorylation of "mTOR, PI3K and AKT proteins"

4. Western Blotting:

The method of "Western Blotting" is dealing with detection, analysis and quantification of the proteins from assay sample. It is well-established analytical method. The method of "Western Blotting" is widely used for the detection of specific-protein-molecules from the tissue-homogenates and cell-lysates. It involves separation of protein through the "Gel Electrophoresis" and followed by the process to transfer to a membrane entitled, "Polyvinylidene Difluoride (PVDF) or membrane of nitrocellulose. For the purpose of visualization, after the transfer of the proteins, there is a method of staining. Finally, the expected proteins are directly identified through N-terminal-sequencing, mass-spectrometry or immunodetection. In the method of "Western Botting", the identification of the proteins is carried through abilities of binding to specific antibodies. Typically, a primary antibody is used in combination with the "Horseradish Peroxidase" (HRP)- conjugated secondary antibody or "Alkaline Phosphatase" (AP)-conjugated secondary antibody for the purpose of detection through the method of chemiluminescent or the method of colorimetry. As alternative method, for direct visualization, one may also use a fluorescently-labelled primary-antibody or secondary-antibody. The method of "Western Blotting" is well recognized for extensive use in biochemistry. It is going to serve the purpose of detection of presence of specific-proteins, determination of the extents of modifications in post-translation, verification of the expression of proteins in cloning applications, analysis of the protein and expression-levels of biomarker, mapping of the epitope of the antibody and testing markers of diseases in clinical setting. The reason for improvement in the sensitivity and the speed of the "Western – Blotting" lies in the need of simultaneous analysis of more proteins in limited samples.

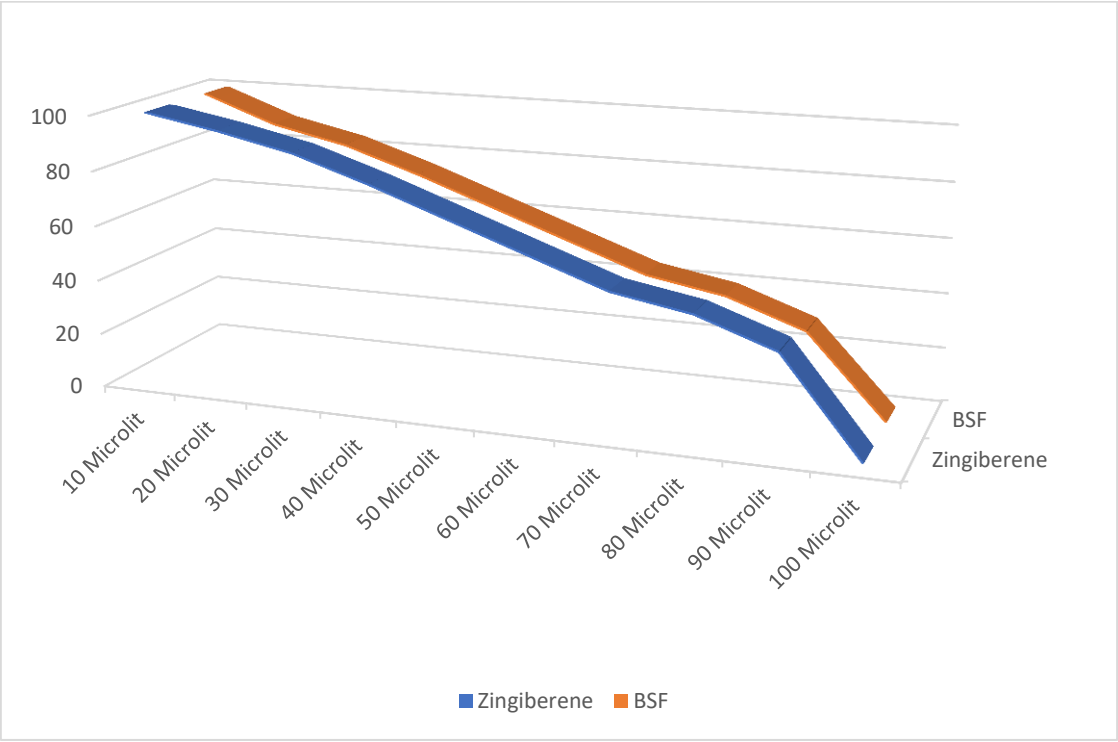



Figure 1. Influence of Methanol Solution of Zingiberene and BSF Powder on Viability of Cells of "Colon Cancer Cell-lines" (Colo 786) belong to Norwegian rat, *Rattus norvegicus* (L).

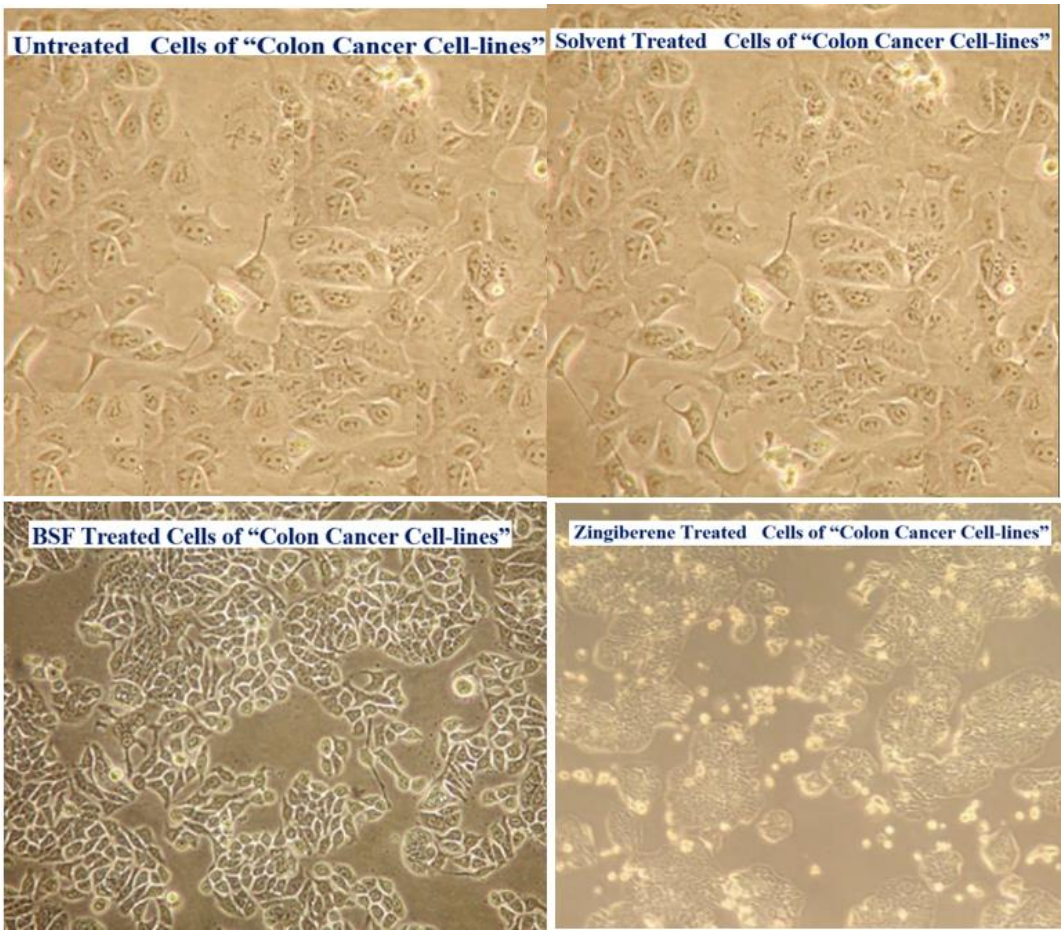



Fig.2: Influence of Methanol Solution (100 Microliters of 100 PPM strength) of Zingiberene and BSF Powder on Cellular Morphology of the Cells of "Colon Cancer Cell-lines" (Colo 786) belong to Norwegian rat, *Rattus norvegicus* (L).

**Fig.3: Influence of Methanol Solution (100 PPM) of Zingiberene and BSF Powder on Tumor Weight (Grams) in xenografted Norwegian rat, *Rattus norvegicus* (L).**

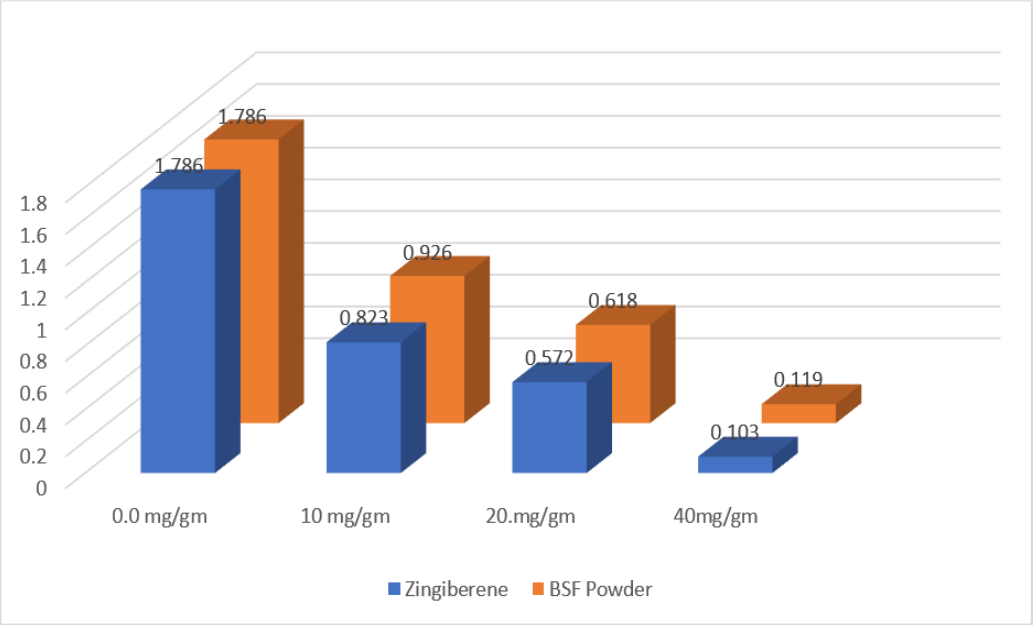
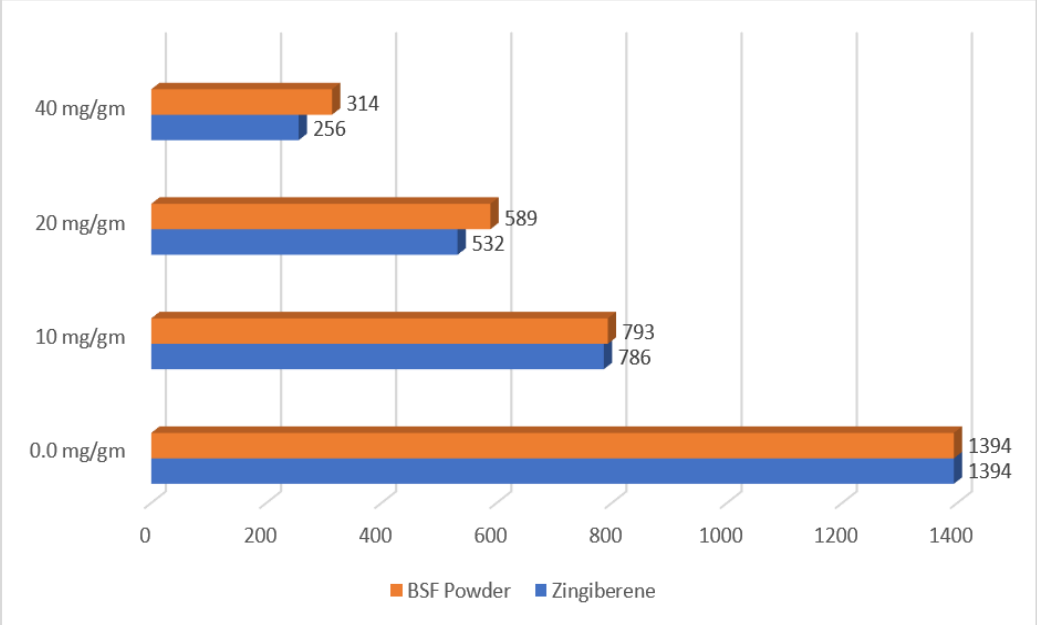



**Fig.4: Influence of Methanol Solution (100 PPM) of Zingiberene and BSF Powder on Tumor Volume (Cubic millimeters) in xenografted Norwegian rat, *Rattus norvegicus* (L).**




**Conclusion**

Zingiberene and BSF powder exerted growth inhibition for colon cancer cell line. The present attempt is reporting zero toxic influence of both, zingiberene and BSF powder in colon cancer affected in Norwegian rat, *Rattus norvegicus* (L). Further, the present attempt confirmed the morphological changes (alteration in the morphology) and formation of colony by the colon cancer cell line. The previous attempts by other researchers have also proved the inhibition of growth of cancer cells by zingiberene, the significant constituents of *Zingiber officinale* (L). The process of autophagy is a vital process. It is helping in the removal of cellular products of dysfunctional categories. It is also expert in the

process of elimination of the cells of abnormal categories from the body. There are several sesquiterpenes, including "Zerumbone" exhibiting potential in the process of inducing autophagy. The "Zerumbone" is a natural sesquiterpene. It is reported to induce autophagy in the cells of cancer of human prostate gland. The cell counting assay was utilized for assessment of rate of proliferation of the cells of colon cancer. Through the use of transmission electron microscopy, the detection of autophagy was carried out. The varied concentrations of monocyclic-sesquiterpene, zingiberene and the methanol maceratives of pre-pupal stages of B.S.F. *Hermetia illucens* (L.) used for treating the "Transfected cells" derived from rat colon include: 000.00 microliters; 100.00 microliters, 150.00 microliters and 200.00 microliters. The fluorescent microscopy was utilized for monitoring. The method of flow cytometry was used for analysis of cell cycle. Expression of the protein was carried out through the immunoblotting. The zingiberene (Terpene of "Monocyclic-Sesquiterpene" class), significant constituent of *Zingiber officinale* (L.) and the methanol maceratives of pre-pupal stages of B.S.F. *Hermetia illucens* (L.) were found considerably inhibiting the proliferation of cancer cells of colon from Norwegian rat, *Rattus norvegicus* (L). Induction of autophagy through the zingiberene and the methanol maceratives of pre-pupal stages of B.S.F. *Hermetia illucens* (L.) is the possibilities for inhibition of growth of cancer cells in the colon of Norwegian rat, *Rattus norvegicus* (L). the methanol maceratives of pre-pupal stages of B.S.F. *Hermetia illucens* (L.) may be utilized for establishment of therapy to control the cancer of colon.

**References**


1. Butler MS. Natural products to drugs: natural product derived compounds in clinical trials. NAT Prod Rep 2008; 25:475-516.
2. Cragg GM, Newman DJ. Antineoplastic agents from natural sources: Achievements and future directions. Expert Opin Investig Drugs 2000; 9:2783-97.
3. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: sources of industrial and medicinal materials. Science 1985; 228:1154-60.
4. Zhang S, Won YK, Ong CN, Shen HM. Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. Cur Med Chem Anticancer Agents 2005; 5:239-49.
5. Kreuger MR, Grootjans S, Biavatti MW, Vandenabeele P, D'Herde K. Sesquiterpene lactones as drugs with multiple targets in cancer treatment: focus on parthenolide. Anticancer Drugs 2012; 23:883-96.
6. Ghantous A, Gali-Muhtasib H, Vuorela H, Saliba NA, Darwiche N. What made sesquiterpene lactones reach cancer clinical trials?. Drug Discov Today 2010; 15:668- 78.
7. Millar JG. Rapid and simple isolation of zingiberene from ginger essential oil. J Nat Prod 1998; 61:1025- 6.
8. Habib SH, Makpol S, Hamid NA, Das S, Ngah WZ, Yusof YA. Ginger extract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics 2008;63: 807-13.
9. Rivenbark, A. G. and Coleman, W. B. (2014). An Introduction to the Conspicuous and Distinguishing Characteristics of Neoplasms. in Pathobiology of Human Disease, 2014A Dynamic Encyclopedia of Disease Mechanisms 2014, Pages 349-366. https://www.sciencedirect.com/science/article/pii/B9780123864567019018.
10. Miyata M; Furukawa M; Takahashi K; Gonzalez FJ; Yamazoe Y (2001). "Mechanism of 7, 12- Dimethylbenz[a]anthracene-Induced Immunotoxicity: Role of Metabolic Activation at the Target Organ". Jpn J Pharmacol. 86: 302– 309. doi:10.1254/jjp.86.302.
11. Sung YM; He G; Fischer, SM (2005). "Lack of Expression of the EP2 but not EP3 Receptor for Prostaglandin E2 Results in Suppression of Skin Tumor Development". Cancer Res. 65: 9304– 9311. doi:10.1158/0008-5472. can- 05-1015. FAO. The State of World Fisheries and Aquaculture 2020; FAO: Rome, Italy, 2020; ISBN 978-92-5-132692-3.
12. Herrero, M.; Wirsenius, S.; Henderson, B.; Rigolot, C.; Thornton, P.; Havlík, P.; de Boer, I.; Gerber, P.J. (2015). Livestock and the environment: What have we learned in the past decade? Annu. Rev. Environ. Resour. 2015, 40, 177–202.