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EVALUATION OF ANTICANCER ACTIVITY OF FERMENTED BARLEY AGAINST SK-OV-3 HUMAN OVARIAN CANCER CELLS

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Abstract : Ovarian cancer is a global problem. The diagnosis of ovarian cancer is often made at a late stage, and there is no reliable screening method. Cytoreductive surgery and platinum-based chemotherapy are the standard therapies for newly diagnosed cancer. Chemotherapy, anti-angiogenic drugs, poly(ADP-ribose) polymerase inhibitors, and immunological treatments are all used to treat recurrent cancer. However, it becomes increasingly resistant to chemotherapy which required alternatives. Research is being done on food products to find potential preventative or therapeutic measures. Numerous disorders can be prevented or treated with fermented barley extract and barley extract (*Hordeum vulgare* L.; Bex). In cancer patients, barley has been found to inhibit tumour growth. Its impact on cancer cells, however, is still undetermined. In this study, the impact of barley on the development of SK-OV-3 cancer cells was investigated. MTT tests showed that the fermented extract of barley reduced the viability of Ovarian cancer cells.

IndexTerms *- Hordeum vulgare L,* Ovarian cancer, SK-OV-3, MTT assays

Introduction:

In the world, ovarian cancer is the sixth most frequently diagnosed cancer in women, making up over 4% of all malignancies in women. More women die from ovarian cancer each year than any other cancer of the female reproductive system, making it the second most common gynecologic cancer after cancer of the uterine corpus (Parkin et al., 2005; Sankaranarayanan & Ferlay, 2006). Three cell types—epithelial cells, stromal cells, and germ cells—commonly give rise to benign and malignant ovarian tumours. More than 90% of malignant ovarian tumours in affluent nations are epithelial in origin, while 5% to 6% of tumours are sex cord-stromal tumours and 2% to 3% are germ cell cancers (Sankaranarayanan & Ferlay, 2006).

Having ovarian cancer in the family is one of the biggest risk factors for the disease. According to estimates, 7% of women who develop ovarian cancer have a family history of the illness (Nguyen et al., 1994). It is thought that hormones like progesterone and estrogen contribute to the development of ovarian cancer (Risch, 1998). Other possible risk factors may include Nulliparity, Oral contraceptive use, Oophorectomy, Early age at menarche, Late age at menopause, Infertility, Lactation, Hormone-replacement therapy, Dietary fat, Obesity, Cigarette smoking, Alcohol consumption, etc. The known risk variables do not significantly raise risk or fully explain the variation in this disease's incidence, it is crucial to emphasize. As a result, many of the causes of ovarian cancer are still unknown (Permuth-Wey & Sellers, 2009).

Due to the great sensitivity of ovarian cancer to chemotherapeutic medicines, targeted therapy, and hormone therapy, surgery plays a special role in the diagnosis, staging, and treatment of ovarian cancer. Recurrent ovarian cancer is difficult to treat, and despite significant advancements in treatment choices, there are still

lots of unresolved issues (Jelovac & Armstrong, 2011). Precision medicine alternatives have been developed as a result of these difficulties. Historically, herbal remedies have been used to prevent or treat illnesses like cancer (Ghorbani, 2014). Herbal products have historically been used as traditional medicines, including Ayurvedic medicine, traditional Chinese and Korean medicines, and Kampo remedies. It has been demonstrated that some herbal remedies can prevent or decrease the side effects of cancer treatment (Nishino et al., 2007; Poonthananiwatkul et al., 2015; Xu et al., 2015).

Hordeum vulgare L. (Barley), has the highest concentration of physiologically active chemicals needed by plants and the highest level of dietary fiber. These metabolites have the potential to be beneficial to health when consumed by humans. Few of its extracts have proven to have strong antioxidant benefits when used as dietary supplements for people. These antioxidant effects are principally brought on by the molecules saponarin, lutonarin, and hexacosanol (Byun et al., 2015; Zeng et al., 2018).

Material and methods:

Sample Collection:

Saccharomyces cerevisiae MTCC – 170 collected from Microbial type culture collection and gene bank (MTCC), Chandigadh, India.

Characterization of Isolates:

On the GYE agar plate, colony characteristics were seen, and Gram staining was used to see the morphological characterization.

Maintenance of Pure Culture:

The transferred yeast isolates were stored at 4°C temperature on GYE agar plates with 0.1g/litre streptomycin for preservation. The isolates were subcultured each week.

Preparation of Barley Sprout:

Barley grains that had been visually sorted and washed in distilled water and soaked in a litre of water. The grains were spread out separately on clean jute bags, covered with a damp cotton cloth, and left to germinate for 24 hours after the water had been drained after 10 hours. During a four-hour break, water is sprinkled on the grains to promote germination.

Fermentation of Barley Sprout:

A mixture of 10g of barley sprout powder and 100ml of sterile distilled water was inoculated with 1ml of *Saccharomyces cerevisiae* at an absorbance of 1.0 at 600 nm. Five days at room temperature and 120 rpm of shaking were used for fermentation. To create the non-fermented extract, 100ml of sterile distilled water was combined with barley sprout powder and agitated for five days.

Methanolic extraction of fermented Barley Sprout:

100ml of methanol was introduced following five days of fermentation. After the mixture was concentrated by the evaporation of the solvent, it was extracted for 48 hours under agitation at room temperature and 120 rpm. (Sharma et al., 2018).

Anti-oxidant Activity

FRAP (Ferric ion Reducing/ Antioxidant Power) Assay:

The FRAP assay performed as described by Benzie and Strain (Z. Ben Ahmed et al., 2016). In a 1:1:10 ratio, 2.5 ml of 20 mmol/L FeCl3, 2.5 ml of 10 mmol/L TPTZ solution, and 25 ml of 300.0 mmol/L acetate buffer are combined to create the FRAP reagent. 20ml of FRAP reagent and 0.1ml of the sample were violently mixed together. In the presence of antioxidants, the ferric tripyridyltriazine (FeIII-TPTZ) complex

is reduced to ferrous tripyridyltriazine (FeII-TPTZ) form, which takes on a strong blue hue with a maximum absorption at 593 nm (D. Ahmed et al., 2015).

Anti-Cancer Activity

At the Sanjivani College of Pharmaceutical Education and Research in Maharashtra, India, the anti-cancer efficacy was tested using an MTT assay. An assay called the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used to examine the cytotoxicity in cultivated cells. (Mosmann, 1983). Ovarian cancer cell line SK-OV-3 was planted in 96-well plates with DMEM and 10% FBS. According to the instructions in the kit manual, the cells were cultivated in 96-well plates to form a monolayer at a density of 1105 cells per well when they were 70-80% full after 24 hours of incubation at 37°C in a humidified 5% CO2/air atmosphere (EZcount MTT Cell Assay Kit, PC: CCK003, Hi-Media). The cells were treated with a one-third dilution series of various extract concentrations (0.05, 0.15, 0.46, 1.37, 4.12, 12.35, 37.04, 111.11, 333.33, and 1000g/ml) when the appropriate cell density was obtained, and they were then incubated for an additional 24 hours. The plate was placed back in the oven for a further 4 hours the next day after each well had received 25l of the 5mg/ml MTT solution. To dissolve the formazan crystals, 100 l of a 60:40 combination of DMSO and IPA was added last. The absorbance of the plate at 570 nm was then measured using a 96-well microplate reader (Multiskan Reader CF3, Thermo Scientific). The following formula calculated percentage cell growth inhibition or percentage cytotoxicity:

%Viability = (AT-AB)/(AC-AB)×100

Where AT=Absorbance of treated cells (drug), AB=Absorbance of blank (only media), and AC=Absorbance of control (untreated).

Statistical Analysis of Data

The cytotoxicity data were reported as the IC50 value, calculated through Graph Pad Prism software Ver. 6.01. Linear regression analysis used a 95% confidence limit and R^2 to define dose-response curves. Compute the concentration of chemical agents needed to reduce the absorbance of the formazan by 50% (IC50).

Result and discussion:

Characterization of Isolates

Colony Characteristics of Isolates

Colony characteristics of *Saccharomyces cerevisiae* were noted from GYE agar plate supplemented with streptomycin (0.1gm/liter). The appearance of the selected yeast on GYE was smooth colonies, white in color, and shiny surfaces.

Morphological Characteristics of Isolates

Methylene blue used on representative smears and examined under a magnification of 1000x having an oval, spherical shape, and budding as a means of asexual reproduction.

Antioxidant activity:

Antioxidants are substances that defend living cells from the harm caused by unstable molecules known as free radicals. Antioxidants are renowned to act and stabilize free radicals, thereby preventing harm. Free radical damage might result in the development of cancer (Ronald L. Prior, Xianli Wu, 2005).

FRAP Assay:

The antioxidant capacity of extracts was determined using a FRAP assay. In this assay, ferric ions were reduced to ferrous ions in the presence of an anti-oxidant.

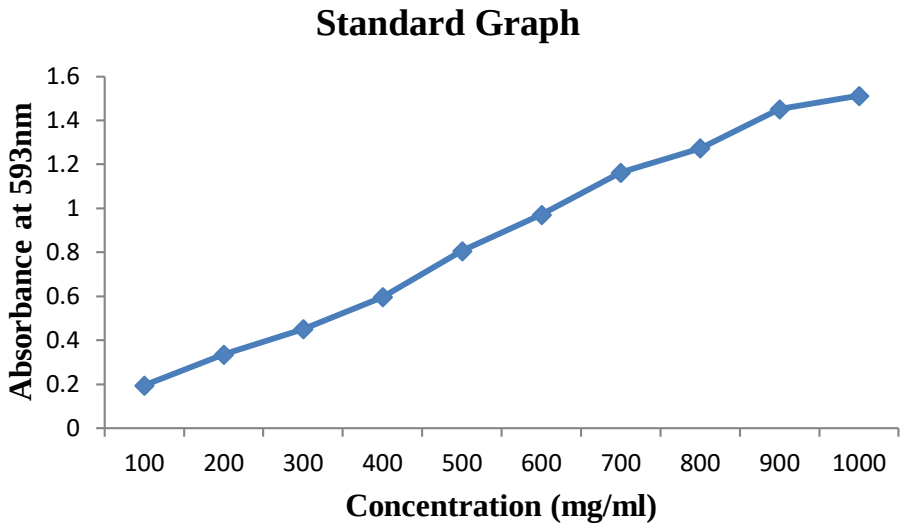

Figure-1: Standard Graph of Ascorbic Acid for FRAP Assay. The X-axis represents Ascorbic Acid Concentration in mg/ml and Y-axis represents Absorbance at 593nm.

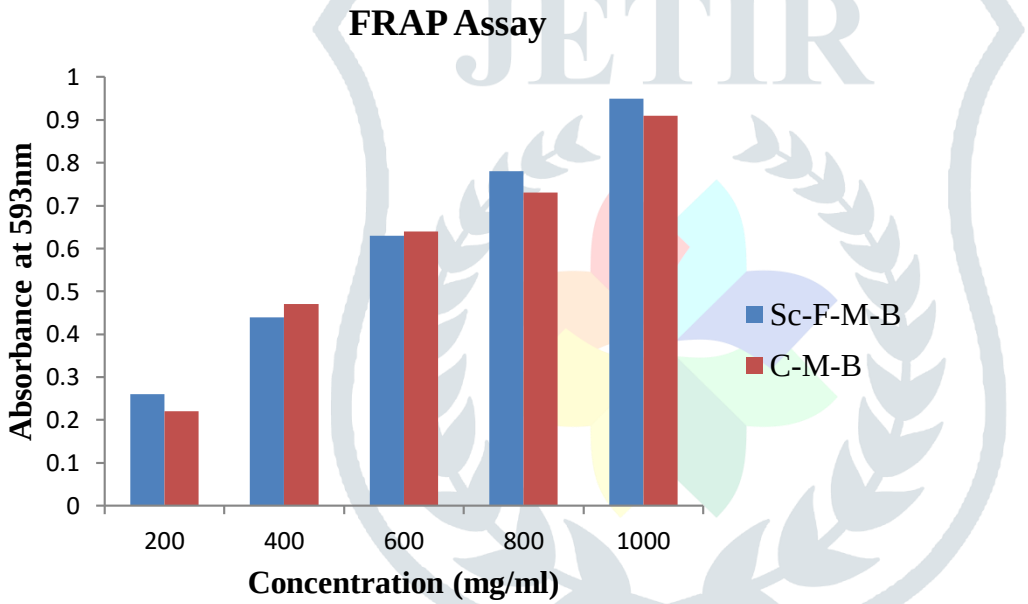

Figure-2: FRAP assay for *Saccharomyces cerevisiae* fermented methanolic Barley sprout extract and Non-fermented methanolic Barley sprout extract. The X-axis represents Concentration in mg/ml and Y-axis represents Absorbance at 593nm.

Results from Graph (Fig-2) show the FRAP assay which indicates *Saccharomyces cerevisiae* fermented methanolic Barley sprout extract gives higher antioxidant capacity having 0.95 absorbances as compared to Non-fermented methanolic Barley sprout extract having absorbance of 0.91. Potent antioxidant activity of Barley was detected by ORAS assay and CAA assay (Zhu et al., 2015).

Anti-cancer Activity:

In the present study, *Saccharomyces cerevisiae* fermented methanolic extract of Barley sprouts, and non-fermented methanolic extract of Barley sprouts was evaluated for in-vitro cytotoxicity assay against SK-OV-3 (Ovarian cancer) cell line. The cytotoxicity assay was carried out by MTT assay.

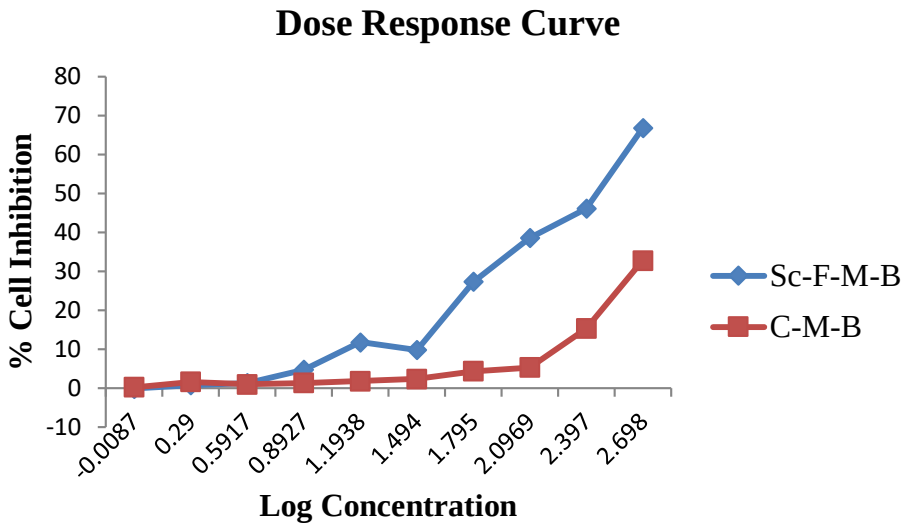

Figure-3: Dose Response Curve of *Saccharomyces cerevisiae* fermented methanolic extract of Barley sprout and Non-fermented methanolic extract of Barley against SK-OV-3 cell line.

Results from Graph (Fig-3) represent the Dose-Response Curve against the SK-OV-3 cell line which indicates *Saccharomyces cerevisiae* fermented methanolic extract of Barley sprout has the highest anticancer activity (IC_{50} value 161.7mg/ml, R^2 value 0.9817), as compared to Non-fermented methanolic extract of Barley sprout (IC_{50} value > 803mg/ml, R^2 value 0.9873). Stefania Nobili et al. found similar results showing that beta-gluten present in Barley protects against heart diseases and Some cancer (Nobili et al., 2009).

Conclusion:

According to the study's findings, the SK-OV-3 cell line was significantly impacted by the fermented methanolic extract of barley. The current study is regarded as a pilot study that opened the door for a subsequent investigation into potential mechanisms and modalities of action on human ovarian cancer cell lines. The anti-cancer activity study revealed that after 24 hours of treatment, non-fermented methanolic barley sprout outperformed fermented methanolic barley sprout in terms of strong antioxidant and severe cell cytotoxicity in the SK-OV-3 cell line. The Saccharomyces cerevisiae fermented methanolic extract of barley may be a suitable candidate for treating ovarian cancer, suggesting that fermentation can boost a plant's capabilities. More research is required in this area.

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