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Free radical scavenging activity and cytotoxicity study of fermented oats (*Avena sativa*)

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ABSTRACT

Oats (*Avena sativa*) is a cereal crop of utmost significance with rich therapeutic and nutritive value. Bioactive substances like tocopherols, phenolic acids, alkylresorcinols, beta-glucan, and avenanthramides present in *Avena sativa* significantly contribute towards its medicinal action. The current research study aims to assess the antioxidant and anti-cancer efficiency of fermented (FO) and non-fermented (NFO) samples of *Avena sativa*. *In vitro* anticancer studies on oats were assessed using colon malignant growth cell lines (HT29) by MTT assay. *In vitro* studies revealed that fermented and non-fermented oats displayed higher antioxidant activity, having a corresponding IC_{50} value of 201.03 μL and 236.46 μL, respectively. The cancer cell death percentage at 250 μg/mL concentration ranged between 58.19% and 51.85%, respectively.

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INTRODUCTION

Oats has been recognized as a salubrious cereal containing high levels of soluble fibre (beta-glucan), protein, lipids, vitamins, antioxidants, phenolic compounds, and minerals. Oats as a functional food has physiological benefits in reducing hyperglycaemia, hyperinsulinemia, hypercholesterolemia, hypertension, and cancer (Adom *et al.*, 2005). Phenolic acids, beta-glucan, tocopherols, avenanthramides, etc., contributes towards the antioxidant activity of oats (Emmons *et al.*, 1999). All these phenolic compounds possess potential health-promoting properties because of their membrane-modulating effects. β-glucans, a soluble dietary fibre present in oats, also exhibit an antioxidant capacity against free radicals (Sridevi *et al.*, 2010).

Anticancer activity is the effect of natural, synthetic, or biological agents that suppress and prevent carcinogenic progression. Several synthetic agents plant-derived chemotherapeutic drugs are being used in the treatment of cancer (Sunderam *et al.*, 2019). Oats contain more than 20 unique polyphenols, avenanthramides exhibiting anti-inflammatory, and anti-proliferative activity, which inhibits the progression of cancer (Meydani, 2009). The primary component of oats encompasses a class of polysaccharides identified as beta-D-glucan, which produces immune responses by activating the monocytes/macrophages (Daou and

Zhang, 2012). Antitumor and anticancer effect of beta-glucan helps in the adaptation of the immune cells and other components of the innate immune system (Hong *et al.*, 2004). The antitumor killing mechanism of beta-glucan is mainly anchored by the neutrophils, primed with betafection (Haas *et al.*, 2009). The current study is to assess the antioxidant activity of *Avena sativa* in fermented (in the presence of *Lactobacillus acidophilus*) and non-fermented samples. In addition, anticancer activity was performed using a colon cancer cell line (HT29).

MATERIALS AND METHODS

Sample Collection

The oats were purchased from stores, cleaned to remove the impurities. They were ground to a fine powder and preserved in a sealed container maintained at room temperature. One gram of finely powdered oats were taken with 50ml of water (in the proportion of 1:50) and autoclaved for 45 minutes. The sample was stored in 4°C for further use. 100μl of *Lactobacillus acidophilus* was added for the preparation of fermented oats. The conical flask was plugged with cotton and was left undisturbed for a time period of 72 hours at room temperature. Finally, both sample extracts were filtered, and the supernatants were collected in separate beakers.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

Radical scavenging activity of the aqueous extracts of oats was measured using the standard procedure (Ye *et al.*, 2013). The stock solution of the oats sample was prepared using Dimethyl sulfoxide DMSO in the concentration of 1mg/ml. Reaction mixtures were taken in different concentrations (50, 100, 150, 200, and 250 μg/mL), and about 3 ml of a 0.004% methanolic solution of DPPH was added to all the test tubes. The absorbance was measured at 515nm after 30 minutes of dark incubation against the blank (DPPH + methanol), and ascorbic acid was used as the standard. The reduction of the DPPH radical was determined by the decrease in its absorbance at 515 nm. The radical scavenging activity (Inhibition %) was calculated using the formula: Inhibition % = [Ac-As/Ac] X 100, where Ac is the absorbance of the control and As is the absorbance of the sample. Radical scavenging activity of the samples was expressed as IC_{50}, which is the concentration of the sample required to inhibit 50% of DPPH concentration.

RESULTS AND DISCUSSION

Anti-Cancer Activity (MTT Assay)

The Colon cancer cell line (HT29) were plated separately using 96 well plates with the concentration of 1×104cells/well in Dulbecco's Modified Eagle's Medium (DMEM) media containing 10% fetal bovine serum (FBS). The cells were maintained in a CO_2 incubator at 37°C (5% CO_2, 95% air, and 100% relative humidity). The cells were washed with 200 μL of 1X Phosphate Buffer Saline (PBS), and then the cells were treated with various test concentrations of the compound in serum-free media and incubated for 24 hours. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL of 3-[4,5-dimethylthiazol-2-yl] 2,5 diphenyltetrazolium bromide (MTT) in 1X PBS was added to each well and incubated at 37°C for 4 hours. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200μL of PBS. The formed crystals was dissolved with 100 μL of DMSO and thoroughly mixed. The formazan dye turns to purple, blue color. The absorbance was measured at 570 nm using a microplate reader (Meerloo *et al.*, 2013). The Cytotoxicity activity (Inhibition %) was calculated using the formula,

$$Inhibition\ \% \ = \ [Ac - As/Ac] \times 100$$

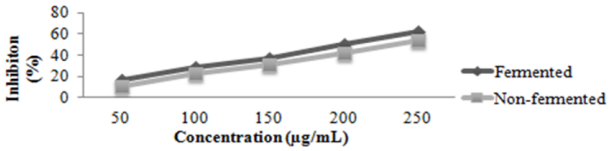

Figure 1: Antioxidant Activity of Fermented and Non-Fermented Oats

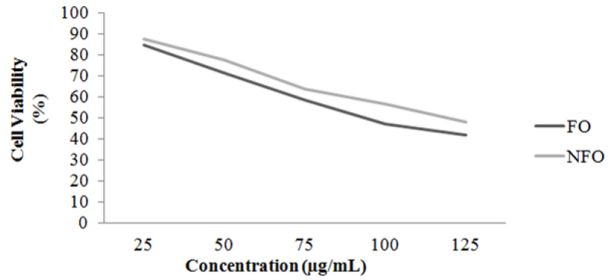

Figure 2: Cell viability of fermented and non-fermented oats in HT29 cells

DPPH Assay

This assay is used to evaluate the free radical scavenging activity of samples based on the drop in the DPPH concentration, a stable free radical (Benzie and Strain, 1999). The results obtained shows the free radical scavenging activity gradually increases along with the concentration of test samples. Fermented oats exhibited greater antioxidant activity

Table 1: DPPH Radical Scavenging Activity of Fermented and Non-Fermented oats

S.No	Concentration (μg/mL)	Inhibition (%)		IC_{50}	
		Fermented	Non- Fermented	Fermented	Non- Fermented
1	50	16.52±1.15	10.46± 0.73	201.85	234.34
2	100	28.29±1.98	22.40±1.56		
3	150	36.65±2.58	31.37±2.19		
4	200	49.73±3.48	42.29±2.96		
5	250	61.73±4.32	53.34±3.73		

Table 2: Cytotoxic activity of Fermented and Non-Fermented Oats on HT29 Cells

S.No	Concentration (μg/mL)	Inhibition (%)		IC_{50} (μg/mL)	
		Fermented	Non- Fermented	Fermented	Non- Fermented
1	25	84.41±5.90	87.48±6.12	64.35	84.41
2	50	71.09±4.97	77.38±5.41		
3	75	58.27±4.07	63.75±4.46		
4	100	47.2±3.30	56.55±3.95		
5	125	41.81±2.92	48.15±3.37		

than non-fermented oats. A comparison of antioxidant activity between fermented oats and non-fermented oats resulted in a p-value > 0.05. IC_{50} values of fermented and non-fermented oats were 201.03 and 236.46, respectively, indicating 50% of scavenging activity. *Lactobacillus acidophilus* and yeast improve the quality of the fermented products (Ak and Gülçin, 2008).

The fermented product exhibited 55.71% radical scavenging activity, whereas the control sample (non-fermented cereal product) recorded a scavenging activity of 40.83% (Figure 1 and Table 1).

A number of cases exhibit that an oat-containing diet enhances the antioxidant capacity. This is due to the presence of bioactive components like Vitamin E, phytic acid, flavonoids, phenolic compounds, sterols, and avenanthramides. The antioxidant compounds are concentrated in the periphery of the kernel (Bajpai and Chaudhary, 2015). A study reported that four beta-glucan hydrocolloids isolated from oats exhibited a significant amount of antioxidant activity determined by the DPPH method (Hastings and Kenealey, 2017).

In vitro Cytotoxicity Activity

The anticancer activity of samples was determined using the MTT assay against the colon cancer cell line (HT29). The mitochondrial activity of living cells is determined based on the conversion of tetrazolium salt MTT into formazan crystals. The concentration of the test sample increases, cell viability decreases. Fermented oats showed lower cell viability than non-fermented oats. IC_{50} values of fermented and non-fermented oats were 64.35 and 88.41, respectively, indicates 50% of cell viability decrease (Figure 2 and Table 2).

Avenanthramides are bioactive compounds, found exclusively in oats and have shown anticancer property against breast cancer cell lines (MDA-MB-231) estimated by MTT colourimetric assays (Razali *et al.*, 2008). Avenanthramides decreases the functionality of breast cancer cells in time and concentration a reliant manner. A similar study reported that avenanthramides isolated from oats showed anti-proliferative action against cancerous human colon cell lines. Several systematic reviews of case-controlled studies suggest that high fibre content can enhance the gut environment conditions by carcinogens-dilution in the colon and decrease transfer time, which might contribute to this form of fortification. Of late, a large, population-based study showed that after further fine-tuning for cereal fibre, the intake of whole grains decreased the danger of colon cancer by 25% (Pašić *et al.*, 2008). Anti-cancer properties of low molecular weight beta-glucan have been investigated against Me45 (melanoma cell lines), A431 (human epidermal carcinoma cells), normal HaCaT (human epidermal keratinocytes) and murine macrophages P388/D1 (Reddy *et al.*,

2000). Low molecular weight beta-glucan from oats significantly decreased cancer cells viability with increased concentration (Choromanska *et al.*, 2015).

CONCLUSIONS

The outcome of the present study reveals fermented and non-fermented oats as an accessible source of natural antioxidants with considerable health benefits. Oats may serve as an excellent lead for the development of an anti-cancer drug against colon cancer. These results suggest future delivery studies on animals with fermented oats for cancer therapy.

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