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Original Article

EVALUATION OF HEAMAGGLUTINATION AND ANTI-CANCER POTENTIAL FROM INDIAN DIETARY PLANTS

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ABSTRACT

Objective: To evaluate the agglutination and anti-cancer activity of different dietary plants, commonly used in Indian dietary system for its therapeutic applications.

Methods: Sap/phloem exudates from different dietary plants were collected in phosphate buffer saline (PBS) was evaluated for lectin activity by using haemagglutination method in serial dilution manner. Anticancer activity of the different samples was evaluated against Ehrlich ascites carcinoma (EAC) cells by using trypan blue exclusion method.

Results: Samples belongs to Cucurbitaceae family showed promising lectin activity among test samples. *Praecitrullus fistulosus* and *Cucumis prophetarum* give 256HU/mg whereas *Cucumis dipsauses* gives 128HU/mg activity, whereas *Abelmoschus esculentus* (16HU) which gives low lectin activity. *Praecitrullus fistulosus* shows anticancer activity 67.38% inhibition at 200µg concentration followed by *Cucumis prophetum* of 56.36% at 200µg concentration against Ehrlich ascites carcinoma (EAC) cells.

Conclusion: The present study demonstrated that dietary lectin having a potential role in the biological field. High lectin activity demonstrates the anticancer effect in Ehrlich ascites carcinoma (EAC) model system, *in vitro.* Further study needs to isolate and evaluate biologically active lectin molecule in order to demonstrate the anticancer effect.

Keywords: Lectin, Dietary source, Agglutination, etc

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INTRODUCTION

Lectins from plants are considered to be one of the major sources lectins which localized in various parts of plants [1]. However, numerous plant-based lectins have been well studied, but their physiological role of these lectins is poorly understood and it is argued that lectins which exhibit biological role need careful study in order to execute its molecular mechanism in the physiological conditions. Lectins found in dietary sources have thought to important part of the human diet. Dietary lectin represents an unavoidable constituent of our diet. These dietary lectins can cause harmful and beneficiary effects in human beings [2]. These lectins performed a wide variety of physiological functions including agglutination, immunomodulation, anti-inflammatory, anti-cancer and so on [3]. Intake of raw or cooked plant material containing lectins can travel through digestive tract from mouth to colon with a substantial part of the molecule remaining in the intact form [4]. These lectins exhibit resistance to the digestive process [5] and it seems to be biologically active throughout the alimentary canal and they perform any activities therein could be modified by inhibitory sugars in the diet [6]. Immunoglobulin-A (IgA) present in the human saliva may also neutralize its effect [7-8]. Although isolated and well-characterized lectins from edible sources, their toxicity upon treatment and their nutritional significance remain unknown. However, intake of purified lectins from the dietary sources and their possible physiological effects still remains unclear.

Cancer is a second leading disease which causes death every year and millions of people are diagnosed with the disease around the globe [9]. Due to adverse effects and complications, currently available chemotherapeutic agents have limitations in treatment. To overcome this problem, phytoconstituents from the plants exhibiting anti-cancer property are emerging as a tool in the prevention of tumor development [10]. Over more than 30% of cancer death was directly associated with the dietary food habits. This is due to the changes in dietary and adaptation of sedentary lifestyle and the burden of this disease are gradually increasing every day [11]. Various research groups were involved and put their effort in order to develop a new therapeutics against cancer by targeting multiple targets in a cellular system. Various experimental studies have demonstrated the cancer preventive effects of different lectins derived from these sources, which attains the greatest prospects in targeting cancer cells. Lectins are considered as one of the promising tools in order to treat and diagnose cancer. Lectins exhibit an anti-cancer property in various cancer cells by activating death pathway such as apoptosis and autophagy [12]. Earlier studies show that pea lectin induces apoptosis in Ehrlich ascites carcinoma (EAC) cells by arresting cell cycle at the G2/M phase and activate apoptosis pathway, *in vivo* [13]. In continuation of our research in identifying and developing anticancer molecules from medicinal plants, herein we screened the dietary plants for lectin and anticancer activity [14].

In this report, we screened the dietary plant's exudates in order to determine the presence of lectin as well as the anti-cancer potential to carry out the further work in future for identifying novel and potent molecule to combat against cancer.

MATERIALS AND METHODS

Chemicals and reagents

Potassium dihydrogen phosphate (KH_2PO_4), Potassium mano-hydrogen phosphate (K_2HPO_4), Sodium chloride (NaCl), Copper sulphate (CuSO4) procured from SRL (Sisco research laboratories). FC reagent procured from Fischer scientific. Trypan Blue was procured from Sigma-Aldrich.

Collection of plant materials

Samples were collected from local area farmers, Mysore and authenticated by Dr. Sharvani K. A, Assistant professor, Department of Botany, Yuvraj's College, University of Mysore, Mysuru. The Herbarium (Accession number: YCM(UOM)0266) was prepared and deposited at Department of Botany, Yuvraj's College, University of Mysore, Mysuru, Mysore.

Preparation of extract

The sap/phloem exudates from plants by incision with a sharp needle and oozing exudates were collected in a sterile tube containing cold 10 mmol phosphate buffer saline (PBS) and immediately transferred to a freezer at-20 °C. Later the samples were removed from the freezer and centrifuged at 12,000 rpm for 15 min. clear supernatant was transferred to a fresh tube and repeat the same procedure until getting a clear solution. The sample free from the debris was subjected to lyophilization, used for the further assay. The samples were named as follows, *Cucumis dipsauses* (S1-), *Lagenaria siceraria* (S2), *Brassica oleracea gongylodes* (S3), *Abelmoschus esculentus* (S4), *Cucumis prophetarum* (S5), *Praecitrullus fistulosus* (S6), *Averrhoa carambola* (S7) and *Actinidia deliciosa* (S8).

Estimation of protein concentration by Lowry's method

Protein concentration was determined as described earlier [15]. Briefly, 0.5 ml of phloem exudates were incubated with the Lowry reagent for 10 min at 37 °C. 0.5 ml of FC (Folin-Ciocalteu) reagent (1:1) was added to reaction mixture and incubates the samples for 30 min at room temperature, and absorbance was read spectrophotometrically.

Preparation of 2% red blood cells (RBC's) cells

The blood was drawn from a healthy volunteer in a fresh tube containing Alsever's solution. Immediately mixture was centrifuged at 2000 rpm for 10 min and transferred the pellet into the fresh tube containing hypo saline solution. Centrifuge the mixture for 10 min at 2000 rpm and repeat the above step until getting the clear supernatant. The obtained packed RBC's cells were used for preparing 2% RBC in PBS solution.

Haemagglutination activity

Agglutination assay was performed by using 96 well plates according to the method described earlier [16]. Briefly, samples (1 mg/ml) were two-fold serially diluted by using PBS in 96 well plates and 2% of RBC's were added to each well and incubate the plate at 37 °C for 60 min and photographed. The haemagglutination activity was measured/expressed in terms of titer. Haemagglutination titer is defined as the minimum amount of protein required to form agglutination id referred as 1titer value.

Evaluation of different dietary lectins samples for anticancer activity

Ethical statement

Mice were maintained as per the principles and guidelines of the Animal Ethical Committee for animal care, University of Mysore in accordance with Indian National Law on animal care and use. The experimental design and study were approved by Institutional Animal Ethics Committee (IAEC) (Ref No: UOM/IAEC/10/2017), University of Mysore, Mysore, Karnataka, India.

Preparation of ehrlich ascites carcinoma (EAC) cells

EAC cells resemble human tumors, which are originally hyperdiploid, rapid proliferative, high transplantable capacity, short life-span and 100% malignancy. Ehrlich Ascites Carcinoma (EAC) cells (5×10^6cells/mice) were injected into the intraperitoneal cavity of the mice. The growth of tumor was monitored every day and recorded until the end of the experiment. Ehrlich Ascites Carcinoma (EAC) cells begin their exponential growth from the seventh day after the tumor cell injection and the animal succumbed to the ascites tumor burden on day 16–20 after injection.

The cells were washed with RBC's lysis buffer to remove RBC's contaminant and followed by saline until getting clear supernatant. The obtained cells were suspended in a culture flask containing RPMI 1640 media.

Tryphan blue exclusion method

Cytotoxic effect of lectin was determined as described earlier [17]. Briefly, a fixed number of Ehrlich ascites carcinoma (EAC) cells was seeded ($0.5x10^6$ cells) in each 6-well plates and exposed the cells to different lectin samples (100µg and 200µg). Cytotoxic effect of different lectin samples was evaluated after 24 h by trypan blue exclusion assay and plotted as described earlier [18].

Statistical analysis

The experiments were carried out in triplicates (n=3) and analyzed by one-way ANOVA (GraphPad Prism version 5.1). Values were expressed as mean±SD.

Table 1: Screening of dietary lectins for agglutination activity (n=3)

S. No.	Plant species	Family	Titer value (HU/mg)
S1	*Cucumis dipsauses*	*Cucurbitaceae*	128
S2	*Lagenaria siceraria*	*Cucurbitaceae*	128
S3	*Abelmoschus esculentus*	Malvaceae	16
S4	*Brassica oleracea gongylodes*	*Brassicaceae*	32
S5	*Actinidia deliciosa*	Actinidiaceae	64
S6	*Praecitrullus fistulosa*	*Cucurbitaceae*	256
S7	*Cucumis prophetarum*	*Cucurbitaceae*	256
S8	*Averrhoa carambola*	*Oxalidaceae*	64

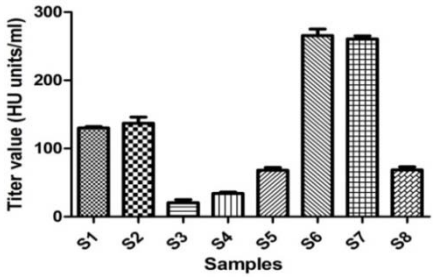

Fig. 1: Screening of dietary plants for lectin activity: A) Two-fold serial dilution of different lectin sample (1 mg) was incubated with 2% trypsinized rabbit RBC's cells and incubated for 1Hr at 37 ° C. visible agglutination was recorded. 10 mmol phosphate buffer saline (PBS) was used as a negative control. Titer values were recorded as mean±SD. The experiments were repeated thrice for each sample (n=3)

RESULTS

Eight samples were used to screen for the presence of lectin activity in their phloem exudates using haemagglutination assay by using rabbit erythrocytes. The phloem exudates *Praecitrullus fistulosus* and *Cucumis prophetarum* exhibit high lectin activity of 256HU/mg at 200µg followed by *Cucumis dipsauses* which give 128HU/mg titer (table 1, fig. 1). Dietary lectins from plant origins gain an attention due to its promising anti-cancer tools in the field of cancer biology. Cytotoxic effect of different lectin sources was determined by using Ehrlich ascites carcinoma (EAC) cells *in vitro*. Fixed number of Ehrlich ascites carcinoma (EAC) cells were exposed to different lectin samples for 24 hr and cytotoxic potential was determined by trypan blue exclusion method. Results exhibit the highest cytotoxic potential observes *Praecitrullus fistulosus* and *Cucumis dipsauses*, up to 67.38% and 56.36% at 200µg followed by other lectins sample as shown in fig. 2. Other lectin samples show moderate anti-cancer activity and their inhibition was found to be *Cucumis dipsauses* (45.08%), *Lagenaria siceraria* (53.59%), *Abelmoschus esculentus*

(20.33%), *Brassica oleracea gongylodes* (44.99), *Averrhoa carambola* (43.78%) and *Actinidia deliciosa* (34.76%) at 200µg concentration.

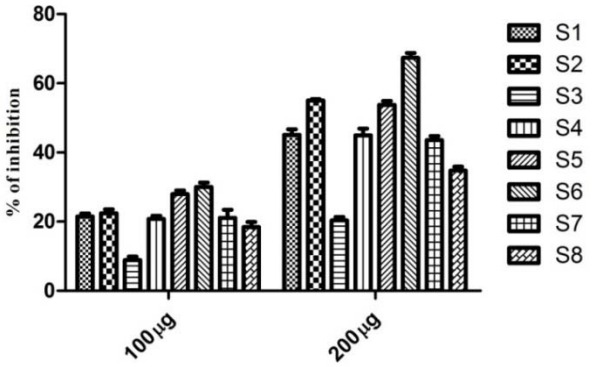

Fig. 2: Screening of dietary plants for anti-cancer activity: fixed numbers of cells (0.2x10^5 cells) were exposed to different lectin sample at 100 µg and 200µg for 24hr and cytotoxicity was assessed by trypan blue exclusion method by using hemocytometer. 10 mmol phosphate buffer saline (PBS) was used as negative control. The cytotoxic potential of each lectin sample was expressed as mean±SD (n=3)

DISCUSSION

For many years, it was believed that lectins are a toxic substance which may harmful to physiological activity. Later in 1970's work led by shows the potential application of lectins in the field of biology [19-21]. According to Dr. Peter J. D'Adamo "eat right for your blood type", which explains the significant effect of lectin in the diet. Lectins present in the sap/phloem exudates which plays an important role in plant defence system as well [22]. In the present investigation, we screened the dietary plant's phloem exudates for lectin activity by using rabbit erythrocytes. The phloem exudates from *Praecitrullus fistulosus, Cucumis prophetarum,* and *Cucumis dipsauses* exhibit high lectin activity. Interestingly all the above-said species belongs to Cucurbitaceae family. Earlier reports showed that presence of lectin in the phloem exudates Cucurbitaceae species supports our findings [23]. The lectin present in the phloem exudates named as phloem protein-2 (PP-2) which acts as a defence mechanism in Cucurbita species. Majority of the phloem exudates lectins were chitin specific, especially chitooligosaccharides specific protein which is reported earlier [24]. But studies on their biological activity in disease care and management is limited. It is urged to explore the biological activity in developing a new therapeutics to combat the various diseases. Among these plants sap of okra plant showed negligible lectin activity, due to the presence of low amount of protein. Further presence of lectin in the seeds of okra was reported [25].

Dietary lectins from plant origin gain an attention due to its promising anti-cancer tools in the field of cancer biology. Lectins were used as diagnosing marker and killing agents in cancer treatment [26]. Cytotoxic effect of different lectin samples was determined by using Ehrlich ascites carcinoma (EAC) cells *in vitro* by trypan blue exclusion method. Again samples from *Praecitrullus fistulosus* possess promising anticancer activity followed *Cucumis prophetarum.* Interestingly, low lectin activity samples also exhibit cytotoxic potential against cancer cells. This may due to the presence of soluble bioactive peptides/proteins and other small molecules which may responsible for the cytotoxic potential of cancer cells [27]. Lectins present in the plant sources acts on cancer cell by recognizing extensive glycosylation level on the cell surface compared to normal cells [28]. This may be the one of the possible mechanism in order to control the cell proliferation and thereby it controls the cancer progression. Some lectins act as immunomodulators and modulate the immune cells and thereby affects the cancer cell proliferation [29]. In the other hand, lectin recognized and binds to cancer cells surface and may internalize through endocytosis or phagocytosis mechanism. The internalized lectin activates the cell death pathways such as apoptosis or autophagy [30]. This induces the cancer cells leads to death and thereby control and kills cancer [13]. Altogether, our preliminary work provides a compressive perspective for elucidating the molecular mechanism of dietary plant lectins that may help in targeting glycosylation pattern and apoptotic pathways for cancer prognosis and therapeutics.

CONCLUSION

The observation in the study reveals the pharmacological significance of the dietary lectins. Lectins from various dietary sources induce agglutination in rabbit erythrocytes by binding to cell surface glycans. *Praecitrullus fistulosus* and *Cucumis prophetarum* have significant lectin activity and comparable with the other lectin sources. Interestingly, *Praecitrullus fistulosus* and *Cucumis prophetarum* posses promising anticancer activity against Ehrlich ascites carcinoma (EAC) cells, *in vitro*. These results confirm the lectins present in the dietary sources was responsible for the anticancer activity against Ehrlich ascites carcinoma (EAC) cells. The lectin-cell interactions and inhibitory activity of cancer cells developing lectin-based cell-based biological response tools in health and disease.

ABBREVIATION

PBS: phosphate buffer saline, HU: haemagglutination unit, EAC: ehrlich ascites carcinoma, PP-2: phloem protein-2, RBC's: red Blood cells, FC: folin-ciocalteu

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AUTHORS CONTRIBUTIONS

Madhu CS designed and perform the work and Dr. Sharada A. C helps to prepare the manuscript and data interpretation.

CONFLICT OF INTERESTS

The Authors declare that there is no conflict of interest

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