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Screening for Oral, rectal and bladder cancer by *Lagerstroemia speciosa* leaves lectin as a marker

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ABSTRACT
*Lectins are among the various active plant components that are considered to minimise the prospects of developing cancer. Various lectins have been tested in vitro mechanisms and have shown anticancer properties thus immense research is still being carried out so that their anticancer properties can be brought to utilisation in vivo as well.The following study is related to examine the patterns of agglutination in ammonium sulphate partially purified Lagerstroemia speciosa leaves lectins along with erythrocytes of normal and Bladder, Rectal, Oral and Leukaemia cancers to determine hemagglutination units. A significant difference was observed in the titer of normal erythrocytes (*40.3±4.90*)and with the erythrocytes of Bladder Rectal, Oral* cancers*(*320.64±27.98*). No significant difference was noted in the Leukaemia(*40.3±4.90*). Hence this study opens a new prospect to look upon to Lagerstroemia speciosa leaves lectin for contemplating it as a marker for cancer in epidemiological screening of Bladder, Rectal and Oral cancer.*
Keywords*: bladder cancer, hemagglutination, Lagersroemia speciosa lectins, marker, oral cancer, rectal cancer*

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INTRODUCTION

There are various regions which are referred as industrial-zones such as Chandrapur, Mumbai, Bhilai, Koraba, Hooghly. These places have various industries like coal mines, chemical factories, tyres, textile, paper mill industries, etc. The residents in these areas face a variety of health issues related to eye, skin, respiratory, digestive and cardiac problems, which get worsen and develop into serious diseases like cancers. The major cause behind these problems are the dumping and disposing of the industrial wastes in the nearby water bodies and its percolation in ground water and in soil. Presence of toxic air pollutants in the environment is another deadly issue for the nearby residents. According to the World Health Organisation (WHO), if cancer is detected at an early stage then the chances of treatment can be momentously improved. Hence it is important to develop convenient and easy mass population screening tests for the residents of such industrial zones. The term screening stands for the examination of healthy populations to separate out the individuals who are in a latent stage of disease without symptoms[1]. Thus, easy and non-invasive cancer markers can be a screen for the risked population. The results of such epidemiological studies will serve as a basis to decide upon the further conduction of precise confirmatory tests for deadly cancers.

New advancements in technologies and the uprising research towards medical diagnosis and treatments are occurring at a fast pace, thus a plethora of new drugs, diagnostic procedures and treatment methods are being developed every year. In the recent years a huge attention has also been given towards cancer and treatments related to it but it is still expected that in the next few decades the number of cancers diagnosed cases will face an increase [2]. In the year 2015, the WHO determined cancer to be the first or second cause of death before 70 years of age in 91 of 172 countries [3]. According to a research the total number of deaths that occurred due to cancer in the year 2018 were 7,84,821[4].These figures necessitate the immense need of new and convenient methods for easy diagnosis of cancer, and it is in

this regard that lectins can be looked upon to serve as a great domain that can be utilised towards cancer diagnosis.

A lectin can be defined as any protein sourced from a plant, animal or microorganism having no immunogenic foundation, possessing the ability to bind with sugar resulting in agglutination activity. Lectins are known to possess the ability to bind to specific carbohydrates present on the cell surfaces of erythrocytes and thus result in agglutination activity with some blood groups. Their ability to bind with polysaccharides, glycoproteins, glycolipids or with free sugars is reversible[2].The most important characteristics of lectins are agglutination and specificity, the former helps to distinguish them from other sugar binding macromolecules. Scientific community is greatly influenced by these binding domains of the lectin and the properties of the amino acids linked with carbohydrate specificity.

The ability of tumour cellsto metastasise is known to be linked with other cells by glycosylation. Malignant transformation of cancerous cells shows alterations in the glycosylation process of the surface glycoproteins. Thus, there occurs a difference in the membrane surfaces between normal cells and cancerous cells. This becomes the basis for the variation in the interaction of lectins with normal and cancerous cells. Cancer is known to proliferate via metastasising into different parts, for which the cancer cells undergo adhesion with the new body cells. Lectins are known to prevent the occurrence of this adhesion as a result, the metastasis of cancer can be stopped[2].

This difference in interaction can be easily observed through the agglutination activities with some lectins. Various lectins have been identified from profuse sources such as wheat germ agglutinin, peanut agglutinin, soybean lectin, jack fruit lectin that abundantly differ from each other in terms of their structure on the molecular basis. The common ground between them is the binding ability to carbohydrates. Lectins have different properties by which they bind to cancer cells and once bound, they show apoptosis, necrosis, autophagy, changes in interleukin production. These changes results in immunological responses with protein kinase activation, which prevents the cancer cell proliferation and causing the inhibition of cancer cell growth [5]. The mechanism followed by lectin to showcase such cytotoxic effects on cancer cells varies from one lectin to the other depending upon the source and the concentration of the lectin [6].

The present study focuses on the *Lagerstroemia speciosa* leaves(LSL) lectin as a preventive cancer marker, because of its relatively different agglutination patterns with normal erythrocytes as compared to the erythrocytes from the considered types of cancers, Bladder (BL), Rectal (RC) and Oral (OR) cancer and Leukaemia (LK). The changes that occur in the cancer cells as compared to the normal cells can be used as the basis for early detection. The property of lectins to recognise glycoconjugates with respect to the carbohydrate group it carries, is being considered for the recognition in markers for cancer. The highly specific LSL lectins is advantageous since it helps to select peptides that get glycosylated and are specially expressed in cancer patients thus widening the windows of cancer diagnosis.

MATERIAL AND METHODS

Lagerstroemia speciosa was chosen for the purpose of isolation of lectin which is found locally growing in India. The plant was authenticated from the Department of Botany of Rashtrasant Tukadoji Maharaj University, Nagpur Maharashtra (Authentication no. LS 1088). The plant belongs to the genus Lagerstroemia and family Lythraceae, is native to southern Asia and is also referred to as the Pride of India. It is a small to medium sized tree with a smooth and flaky bark and grows upto the height of 20 meters tall. The leaves of the plant are oval to elliptical in shape with white to purple flowers, bloom once in a year in summer. The roots, barks and leaves of the plant have been used traditionally in medicines for curing various illness [7].The presence of lectin was confirmed by observing its agglutination with human erythrocytes. Hemagglutination assays for this lectin was performed on erythrocytes from confirmed cases of BL, RC, OR and LK cancer by an oncologist for a comparative evaluation. Similar assays were performed on normal A, B and O blood group assays and the results were analysed to compare them. Detailed information about each participant was obtained through a structured questionnaire. Written consent was taken from each participant after complete oral explanation about the study. The enrolled participants were screened for eligibility using a set of specified inclusion in accordance with residential exposure and exclusion criteria like diabetes patients.

Isolation and Partial purification of Lectin:

*Lagerstroemia speciosa*leaves (LSL) were obtained from a roadside growing plant and the dirt was warded off by cleaning them with water.The homogenised lectins were centrifuged at 7000 rpm and dialysed for 2-3 days against saline. Dialysed samples were partially purified with 30-50, 50-70, 70-90% ammonium sulphate purification. The role of ammonium sulphate is to cause the coagulation of the protein in the extract by absorbing the water of hydration surrounding it. The partially purified lectins were checked for the Hemagglutination Unit(HAU) and specific activity.

RBC preparation:

Whole blood is subjected to 3 washing cycles with 0.9 % saline, each cycle comprising of addition of saline, centrifugation and discarding the supernatant. After the third cycle 200µl of RBC's are taken to make up 2% RBC suspension by 0.9 % saline.

Hemagglutination assay:

The hemagglutination assay was performed in a 96 well round bottom microtiter plate. The wells were subjected to an addition of 100µl of 0.9 % saline, along with the addition of 100µl of lectin in the first column of wells which was serially diluted to last well. The plates were then put through an incubation period of 30 minutes. Further, the RBC suspension of the normal individuals were added in accordance to the respective blood groups, which were compared to the first column taken as control. This was followed by an incubation of one hour on a rotary shaker at 37°C. The similar experiment was performed for the BL, RC, OR and LK cancer RBCs. The assays were observed and analysed microscopically and the titer values were calculated for all.

Statistical analysis

The hemagglutination activity of normal erythrocytes and BL, RC, OR and LK cancer erythrocytes are compared by using independent student's' test. The significant difference in control and cancer cases was considered asp<0.05.

RESULTS

The LSL extracted lectin was used to study the agglutination patterns with erythrocytes of normal individuals(n=50) and study subjects (n=63); BL (n=14), RC (n=12), OR (n=25), LK(n=12) cancer patients. The obtained concentration of the proteinwas0.01 gm/100µl of lectin. Table 1 reveals the protein concentration in different fractions of ammonium sulphate fractionation. Hemagglutination activity of normal erythrocytes and BL, RC, OR cancer and LK are depicted in table2.The HAU for normal erythrocytes (control) and LK was calculated to be 40.3±4.90 and HAU for BL, RC, OR cancer erythrocytes were found to be 320.64±27.98.

Table 1: Protein extraction using ammonium sulphate fractionation

Crude extract Protein (Dialysed)	Ammonium sulphate fraction		
	30-50%	50-70%	70-90%
100 (mg/ml)	30 (mg/ml)	50 (mg/ml)	60 (mg/ml)

Table 2:Comparison of HAU/ml and specific activity in control and cancer erythrocytes

Study Subjects	Number of Cases (n=113)	Protein (mg/ml)	Hemagglutination unit/ml (HAU/ml)	Specific Activity	Statistical comparison Control V/S Cancer patients (P value)
Control	50	100	40.3±4.90	0.4	-
Bladder Cancer	14	100	320.64±27.98	3.2	p<0.0001
Rectal Cancer	12	100	320.64±27.98	3.2	p<0.0001
Oral Cancer	25	100	320.64±27.98	3.2	p<0.0001
Leukaemia	12	100	40.3±4.90	0.4	NS

The HAU/ml is the reciprocal of the minimum concentration of lectin required to cause the agglutination of erythrocytes. Specific activity: (HAU/ml)/mg of protein.

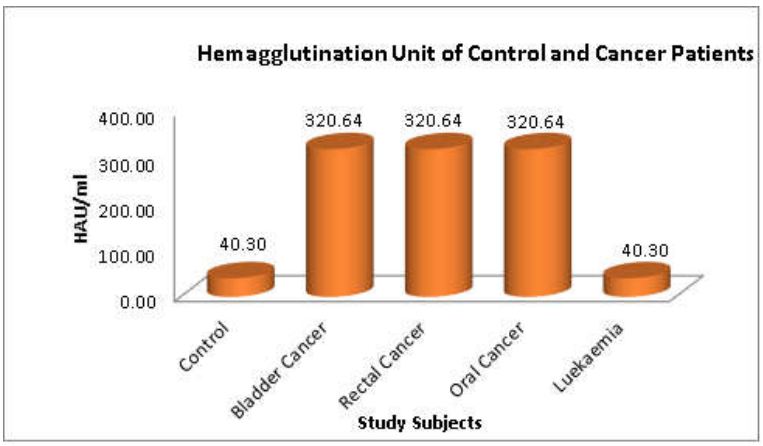

Figure 1: Graphical representation of HAU/ml for BL, RC, OR and LK cancer with normal erythrocytes

DISCUSSION

The LSL were found to contain an arabinose specific lectin (unpublished data). The agglutination of the lectin was studied with erythrocytes of BL, RC, OR and LK cancer samples and compared with normal erythrocytes of A+ve, B+ve and O+ve blood groups. The HAU for BL, RC and OR cancer was increased than the control whereas, the HAU for LK and control is quite similar. It was observed that lesser concentration of the LSL lectin was required for agglutination of BL, RC and OR cancer erythrocytes than required for the normal erythrocytes. At the same time similar concentration of the LSL lectin was required for the agglutination of LK and normal erythrocytes.

Studies in the line with the present study to demonstrate hemagglutination patterns have also been carried out. Ramteke, *et. al.* studied the hemagglutination pattern of galactose specific lectin obtained from *Tridaxprocumbans* plant (leaf TPL-L, stem TPL-S and calyx TPL-C) with the normal erythrocytes and those from breast cancer patients from stages I to II. It was found that lesser dilutions of all the three lectins were required to agglutinate the erythrocytes of breast cancer than in the erythrocytes of normal samples. The hemagglutination units reported by Ramteke *.et. al*. for TPL-L, TPL-S and TPL-C for control samples was found to be 2560,1280 and 2560 respectively and those for breast cancer patients (stages I and II) were found to be 950, 470,950 and (stages III and IV) 400, 200, 400 respectively [8].

Durgawale *et. al.* studied similar pattern using the *Synadium grantii* root (hook F) lectin, a galactose specific lectin for comparative studies between erythrocytes of normal individuals and of different types of cancer. This study reported an increase in the HAU of breast cancer samples thus differing from the results of Archana *et. al.* and no significant increase in the HAU of other types like lung, cervix, stomach etc suggesting an increased receptor sites on the breast cancer erythrocytes [9].The results by Durgawale *et. al.* of increased HAU for breast cancer erythrocytes is similar to the results of the present study of increased HAU for RC cancer erythrocytes. Durgawale, PP.*et. al*. obtained the HAU/ml for rectal cancer cut off range as 80-240 whereas in the present study the HAU/ml for the same was obtained higher titer value as 320, as depicted in Figure 1.

Thus, the present study shows diverging results from those of Ramteke, AP*et. al.* since, more dilutions of LSL lectin was required for agglutinating the BL, RC and OR cancer erythrocytes as compared to the normal erythrocytes. Durgawale *et. al.* on the basis of their results concluded that the number of galactose epitope receptors might have increased in the breast cancer erythrocytes. Hence, linking these conclusions to the present study, it can be said that the BL, RC and OR cancer erythrocytes might have a larger number of arabinose epitope receptors on their surface than those on the normal erythrocytes. Whereas, it may be attributed that there are no differences in the number of arabinose specific receptors on LK and normal erythrocytes [9].

To reach into the deeper insights of lectin studies, the hemagglutination titers can be compared to the cell line studies. Various studies that have been carried out on lectins have depicted the following effects on cancerous cell lines, thus reflecting their cytotoxicity on cancer cells.Kiss, R.*et. al.* compared the effects of five type of lectins on the growth in three types of cell lines of human colorectal cancer. The lectins used were, *Phaseolus vulgaris* (PNA), *Griffonias implicifolia* (GSA), concanavalin A (Con A), wheat germ (WGA), and peanut (PHA-L) agglutinins. They concluded that the lectin type and its concentration influenced the cellular growth. Also, the more was the time exposure and the concentration of lectins, the higher were the effects seen[10].The effects of Viciafaba agglutinin (VFA), a lectin found in broad beans was studied by

Jordinson, *et. al.*on the Colorectal adenocarcinoma derived cell lines (LS174T,17SW1222, and HT29). They shed light upon the therapeutic nature of VFA since it inhibited the cellular proliferation and also slowed down the development of colon cancer [11]. Schaefermeyer *et. al.* analysed the treatment effects of *Viscum album* (Iscador) in patients with different stages of pancreatic cancer and concluded that the patients that were given this treatment showed better quality of survival times[12].Hajto et. al. who studied the same lectin stated increased activities of Natural Killer cells (NK) and Antibody-dependent cell-mediated cytotoxicity (ADCC) along with an increased count of large granular lymphocytes (LGL) in patients with breast cancer[13]. Bhat, *et. al.* studied the *Punicagranatum* (pomegranate) lectin and it was found that it is lactose and arabinose specific, that showed cytotoxicity at a dose of 185.4 µg/ml against MCF-7 cell lines[14].

Thus, the remarkable differences in agglutination suggests a further scope for the consideration of LSL lectin as a marker for the detection of cancer via conducting the titer test procedures of erythrocyte samples. Specially, remarkable differences can be seen in the titer plates of the BL, RC and OR cancer samples as compared to the normal samples that have been considered. The present finding opens doors for future scope to study the behaviour of the LSL lectin with the other types of cancer erythrocyte samples as well, which the present study could not cover, and also can be further broadened towards cancer cell-line experiments to determine the toxicity of the lectin.

CONCLUSION

Taking into consideration the fact that lectins can cause agglutination with cancer cells along with their ability to recognize and bind to discrete carbohydrates, they are being contemplated to be used in formulating cancer markers. The LSL lectin considered in this study can be used as a marker for detection of BL, RC, and OR cancer from the normal populations. This can be done by conducting simple erythrocyte titer tests. However, the fact of whether the LSL lectin can be used on populations to see if it can work as a preventive marker for early detection of cancer must be further studied. There is also a scope of formulating oral drugs derived from lectins, as they show resistivity towards gut digestion and thus, they survive the gut passages thus showing their biological intactness and maintaining their activity when they enter the circulation. A diet plan containing foods rich in non-toxic lectins can be prepared and recommended to cancer societies for the benefits of cancer patients.

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