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RESEARCH ARTICLE

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CROSS CLINICAL REACTIVITY AMONG LIGUSTRUM ROBUSTUM AND CORYLUS MAXIMA POLLINOSIS

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Manuscript Info

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Abstract

Pollen from various tree and grass species is the most common cause of allergy responses around the world. Several proteins found in Ligustrum pollen have previously been identified as allergens that cause sensitization and the onset of allergic symptoms in atopic people.Our study includes the cross-reactivity between pollen extracts of *Ligustrum robustum* with *Corylus maxima*for clinical significance. Immunological and biochemical characterization was carried out for the purified GST compound which was assessed by SDS-PAGE. The Cross-reactivity studies among allergens and relevant species was evaluated by ELISA inhibition assays. In biochemical assays c-GST was not enzymatically active, whereas l-GST demonstrated high enzymatic activity.

Introduction:-

Ligustrum robustum is an invasive, poisonous plant which is implicated as a cause of allergies such as asthma.[1]*Ligustrum* is dark bluish or purplish black berries are eaten and dispersed by birds to cause hay fever[2]and asthma[3], but as the flower is pollinated by insects[4], it is sometimes argued that privet pollen cannot cause these allergies on any significant scale[5].*L. robustum* inhibits tumor cell growth both *in vitro* and *in vivo* by inducing apoptosis in a caspase-dependent way without apparent hepatic toxicity and histological damage[6].The major invasive alien plant species include *Sorghum bicolor*[7], *Parthenium hysterophorus*[8], and*Birch pollinosis*[9].Cor a1 showed amino acid sequence identities[10] with seed storage proteins, *Sesamum indicum*[11-12] (Sesi 3: 57.6% identity). Allergic symptoms to ash pollen can be the consequence of sensitization to cross-reactive allergens from other sources. *Pichia pastoris* yeast[13] is an appropriate system for the efficient production of Cor a1 like allergens, which could be used as analogous allergens and predictors of clinical sensitization. IgE from allergic sera can induce histamine release from basophils and they might play a functional role in the clinical symptoms of allergy[14]. Oxygen radical measurements predicted the severity of pollinosis and IgE receptor related genotype FCER1B was associated with increased oxygen radical generation[15]. Allergenicity of the Sorghum plant in Andhra Pradesh was found to be 54.9%, but the allergens responsible have not been

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characterized well[16].GST levels were always considerably higher in the upper leaves than in the middle and lower leaves, in which the changes were often not significant[17].Ole e1 is a major allergen from olive pollen with an IgE-binding frequency around 80% among allergic population, and cross-reactive allergens have been found in ash, lilac and *Ligustrum*[18].

Material And Methods:-

Purification of GST from *Ligustrum robustum*

Flowers of *Ligustrum* were made into smoothie[19]and GST purification from the extract were performed as per manufacturer instructions (Takara Bio).Eluted protein samples were pooled and filled into a dialysis membrane with a cut-off of 6-8 kDa (Pall Corporation). Protein suspension was dialysed in approximately2 L of dialysis buffer and the buffer was changed for four times against sodium phosphate (Na2HPO4, 50 mM, pH=8.0) buffer to remove cell toxic imidazole[20].

SDS-PAGE analysis of GST proteins

Protein samples along with molecular weight marker were analyzed by means of SDS-PAGE. For visualization, gels were stained with Ezee Blue and subsequently exposed in a gel imager. All gels were prepared and run in a Mini Gel chamber (BioRad, USA). 15 µL of protein sample were mixed with 5 µL of 2x loading dye and were boiled at 95°C in the heating block for 5 min. 3 µL of protein ladder (Fermentas) and the samples were loaded and at a constant voltage of 50V was applied (Bio-Rad, USA) until the dye completely left the gel[21].

Enzymatic activity assay

The enzymatic activity of GST was determined in an enzymatic activity assay (Glutathione-S-transferase assay kit, Abcam, ab65326)[22].10 µl of GST sample from *Ligustrum* extract was diluted in 190 µl of ddH2O and put on ice. 150 µL of assay buffer were added to flat bottom 96 well plates (Puregene). Another 20 µL of glutathione solution were pipetted into the blank and samples. To start the enzymatic reaction, 10 µL of the substrate Benzyl isothiocyanate (BITC) were added and the absorbance was measured immediately at 274 nm in a plate reader (Thermo Multiskan). The absorbance was determined every 5 min over a time period of one hour[23].

Determination of pollinosis by ELISA

ELISA plates were coated each with 100 µL(50 µg/mL) of Cor a1 (Corylus pollen) and VT20(*Ligustrum*pollen)in carbonate buffer overnight at 4°C. Subsequently, the plates were washed twice with PBST (250 µL/well). To avoid unspecific binding, the plates were blocked with 150 µL/well of PBST (1% bovine serum albumin, BSA) for one hour at RT. 100 µL/well of purified IgG and or IgE (1:1000 in PBST; Puregene) were added andincubated for 30 min at 37°C followed by 60 min at 4°C. The plates werewashed and 100 µL/well of goat anti-human IgG and or IgE HRP-linked (1:2000 in PBST; Puregene) were applied for 1 h at 37°C. The absorbance was measured at 405 nm in a plate reader (Thermo Multiskan, USA)[24].

Detection of cross-reactivity among pollen allergens

ELISA plates (Puregene) were coated with 100 µL/well of BPE extract as a positive control (each 50 µg/mL) in carbonate bufferovernight at 4°C or for one hour at 37°C. Subsequently, the plates were washed twice with PBST andblocked with 150 µL/well of PBST along with 1% BSA for 2 hours at RT.100 µL/well of anti-humanIgEandanti-human IgG (1:1000 in PBST; Puregene) were added and incubated for 90 min at 37°C. The plates were washed and 100 µL/well of goat anti-human IgG/IgE HRP-linked (1:2000 in PBST; Puregene) were applied for 1 h at 37°C. The absorbance was measured at 405 nm in a plate reader (Thermo Multiskan, USA)[25].

Results And Discussion:-

Purification of GST from *Ligustrum robustum*

Stained protein bands at a size of 32kDa represent GST produced in satisfactory yields. The pooled pre-purified protein sample was dialysed and were analyzed by SDS-PAGE.The gels were scanned on a Gel scannerwith a white light converter (UVI-Tech, Lark Innovative) and the resulting image wasanalyzed with UVI-Tech Software (Figure).

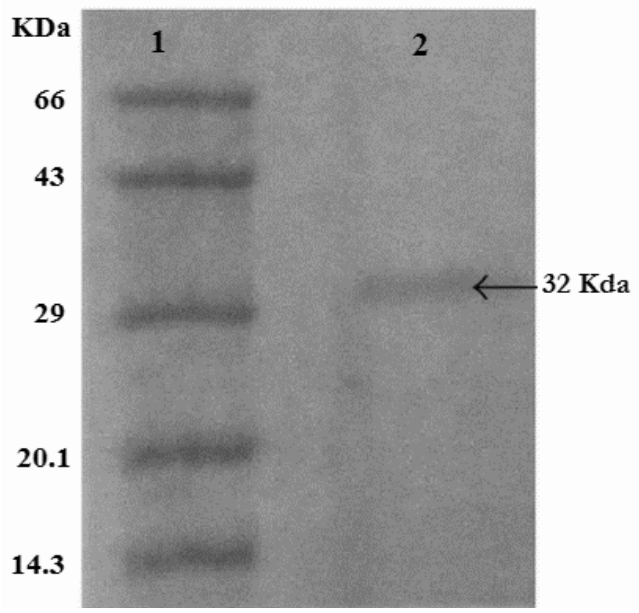

Figure 1:-Analysis of GST protein on 12% SDS-PAGE. Lane 1 represents the protein marker (Genei) and Lane 2 represents the protein band at calculated size of 32KDa.

Enzymatic activity of Ligustrum GST

The enzymatic activity of GST and BPE was investigated in a GST activity assay kit. The results showed that CorylusGST was not enzymatically active when compared to Ligustrum GST, whereas BPE used as positive control displayed high enzymatic activity.GST from the crude extract of purified and used for measurement of total GST activity by a suitable substrate Benzyl isothiocyanate (BITC)at 274nm which is used for the broadest range of GST isozymes.

Sample	A_{274}/min	GST Activity (U/mL)
Corylus GST	83	156
Ligustrum GST	376	812
BPE* (Positive control)	1978	4952

Table 1:- Enzymatic activity of GST measured in GST activity assay kit and expressed in U/mL, respectively. * Bee Pollen Extract

Determination of pollinosis by ELISA

VT20pollen showed a specific IgG response and antibodytitres increased after every regular intervals (Fig). IgEantibodies level did not shown significant raise after incubation neither in the VT20 nor in the Cor a1 group. A moderate increase of IgEantibodytitres was seen in both groups. These findings demonstrated that VT20 was immunogenic and IgG antibodies, slightly upregulated IgE levels were detected. The same applied to the major *Corylus* pollen allergen Cor a1. This fact suggests that VT20 shows similar folding and structure compared to the Cor a1 of *Corylus* pollen.

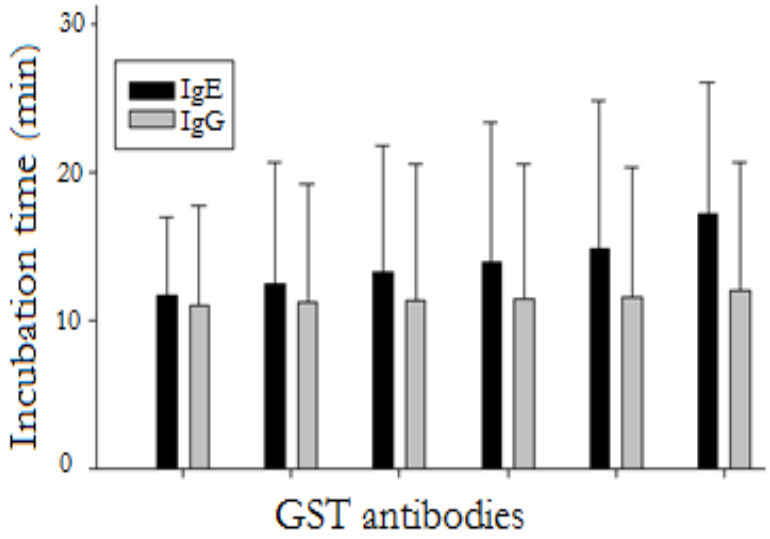

Figure2:-GST antibodies raised towards the VT20 and Cor a1 pollens at different intervals. n = 3 for all groups,values are means ± SE.

Detection of cross-reactive pollen allergens

We investigated a potential cross-reactivity between GST from Corylus and GST from *Ligustrum*. Therefore, ELISA-plates were coated with p-GST extract along with b-GST extract and incubated with IgG and or IgE-antibodies. No cross-reactivity between GST-specific Antibodies and HDM-GST was observed (Fig. white bars). In contrast, GST-specific IgG clearly recognized GST in VT20 (Fig., grey bars).

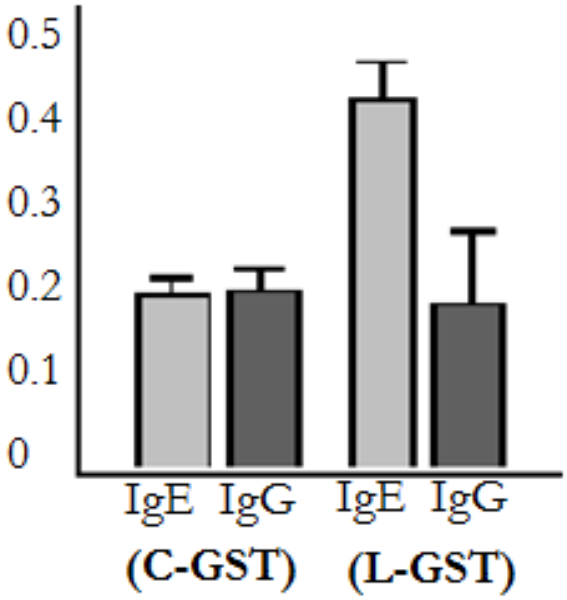

Figure 3:-Detection of GST specific antibodies cross-reacting with Ligustrum pollens, but not seen in Corylus GST.

Conclusion:-

GSTs belong to a well-conserved enzyme superfamily which are, besides several other functions, mainly responsible for the detoxification of potential harmful substrates. Sequence alignments of *Corylus*-GST with several known GSTs revealed a homology of 82% with a protein belonging to the GST omega class. Recently, a GST-like protein has been found in Corylus pollen and was present in high amounts. However, biochemical and immunological properties of *Ligustrum*-GST have not been investigated by now. Most interesting,to date, no data about the allergenicity of GST and its potential role in the development of Ligustrum pollen allergy has been gathered. Hence, our study has attempt to identify the GST levels in *Ligustrum*and the cross-reactivity of antibodies towards the

raised GST levels. However, we showed that it is still a minor allergen for humans. Whether this is due to its release from the pollen or intrinsic properties will be elucidated in future experiments.

References:-

1. Carinanos P, Casares-Porcel M. Urban green zones and related pollen allergy: A review. Some guidelines for designing spaces with low allergy impact. Landscape and urban planning. 2011 Jun 15;101(3):205-14.
2. McGregor PG, Council AR. Prospects for biological control of privet (Ligustrum spp.)(Oleaceae). Landcare Research Contract Report LC9900/127, for Auckland Regional Council. 2000 Jun.
4.Theodorou P, Radzevičiūtė R, Lentendu G, Kahnt B, Husemann M, Bleidorn C, Settele J, Schweiger O, Grosse I, Wubet T, Murray TE. Urban areas as hotspots for bees and pollination but not a panacea for all insects. Nature communications. 2020 Jan 29;11(1):1-3.
5.Barber D, De La Torre F, Lombardero M, Antepara I, Colas C, Dávila I, Tabar AI, Vidal C, Villalba M, Salcedo G, Rodríguez R. Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. Clinical & Experimental Allergy. 2009 Nov;39(11):1764-73.
6.Zuo HJ, Liu S, Yan C, Li LM, Pei XF. In vitro and in vivo evaluation of antitumor activity of Ligustrum robustum, a Chinese herbal tea. Chinese journal of integrative medicine. 2019 Jun;25(6):425-30.
7.El-Kenany ET, El-Darier SM. Suppression effects of Lantana camara L. aqueous extracts on germination efficiency of Phalaris minor Retz. and Sorghum bicolorL.(Moench). Journal of Taibah University for Science. 2013 Apr 1;7(2):64-71.
8.Navie SC, McFadyen RE, Panetta FD, Adkins SW. The biology of Australian weeds. 27. Parthenium hysterophorus L. Plant Protection Quarterly. 1996;11(2):76-88.
9.Taudorf E, Moseholm L. Pollen count, symptom and medicine score in birch pollinosis. International Archives of Allergy and Immunology. 1988;86(2):225-33.
10.Krásný L, Tišerová H, Jonák J, Rejman D, Šanderová H. The identity of the transcription+ 1 position is crucial for changes in gene expression in response to amino acid starvation in Bacillus subtilis. Molecular microbiology. 2008 Jul;69(1):42-54.
11.Beyer K, Bardina L, Grishina G, Sampson HA. Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: seed storage proteins as common food allergens. Journal of Allergy and Clinical Immunology. 2002 Jul 1;110(1):154-9.
13.Palomares O, Swoboda I, Villalba M, Balic N, Spitzauer S, Rodríguez R, Valenta R. The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. International archives of allergy and immunology. 2006;141(2):110-8.
14.Cantani A. Pediatric allergy, asthma and immunology. Springer Science & Business Media; 2008 Jan 23.
15.Ichiishi E, Tada M, Ohta Y, Sami M, Kanda T, Ikeda M, Kohno M. Genomic approaches and oxygen radical measurement as biomarker candidates of off-season predictor of pollinosis: a pilot study. Current Pharmacogenomics and Personalized Medicine (Formerly Current Pharmacogenomics). 2015 Apr 1;13(1):41-50.
16.Veeravilli S, Nimmagadda G, Dondapati K, Naredla K, Bodiga VL. Cross-reactivity studies of Ligustrum robustum with Corylus avellana pollinosis for clinical significance. Drug Invention Today. 2020 Jan 1;13(1).
17.Bencze S, Bamberger Z, Janda T, Balla K, Bedő Z, Veisz O. Drought tolerance in cereals in terms of water retention, photosynthesis and antioxidant enzyme activities. Open Life Sciences. 2011 Jun 1;6(3):376-87.
18.Castro L, Crespo JF, Rodríguez J, Rodríguez R, Villalba M. Immunoproteomic tools are used to identify masked allergens: Ole e 12, an allergenic isoflavone reductase from olive (Olea europaea) pollen. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2015 Dec 1;1854(12):1871-80.
19. Kumar VP, Prasanthi S, Reddy AC, Raj ND, Anudeep L. Characterization studies of thermostable alkaline phosphatase from various plant seeds. Journal of Applied Biosciences. 2010;36:2403-8.
20. Bayer EA, Wilchek M. [14] Protein biotinylation. Methods in enzymology. 1990 Jan 1;184:138-60.
21. Schäfer F, Seip N, Maertens B, Block H, Kubicek J. Purification of GST-tagged proteins. Methods in enzymology. 2015 Jan 1;559:127-39.
22. Siva Kumar V, Prakash R, Murali MR, Devaraj H, Niranjali Devaraj S. In vivo micronucleus assay and GST activity in assessing genotoxicity of plumbagin in Swiss albino mice. Drug and chemical toxicology. 2005 Jan 1;28(4):499-507.
23. Kishimoto A, Ogura T, Esumi H. A pull-down assay for 5'AMP-activated protein kinase activity using the GST-fused protein. Molecular biotechnology. 2006 Jan;32(1):17-21.
24. Vemuri PK, Talluri B, Panangipalli G. Immunological and biochemical characterization of glutathione-s-transferase in Parthenium pollinosis and its cross-reactivity with birch pollinosis for clinical relevance. International Journal of Pharmaceutical Sciences and Research. 2016 Aug 1;7(8):3533.

25. Weber RW. Cross-reactivity of pollen allergens. Current allergy and asthma reports. 2004 Sep;4(5):401-8.