ORIGINAL ARTICLE

Cross Clinical Reactivity among Wild Privet and Gaint Hazel Pollinosis

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Abstract

Background: Pollen from various tree and grass species is the most common cause of allergy responses around the world. **Objectives:** In this study, we focused on the allergenic potential and cross-reactivity between pollen extracts of *Ligustrum robustum* and *Corylus maxima*, aiming to understand the clinical significance of these interactions. 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 *L. robustum* with *C. maxima* for clinical significance. **Methods:** Immunological and biochemical characterization was carried out for the purified GST compound which was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The cross-reactivity studies among allergens and relevant species were evaluated by enzyme-linked immunosorbent assay inhibition assays. **Results:** In biochemical assays, central goods and services tax (c-GST) was not enzymatically active, whereas l-GST demonstrated high enzymatic activity. **Conclusion:** This research contributes to a better understanding of the allergenic properties and cross-reactivity patterns between these two pollen sources. The obtained information could have implications for the development of diagnostic and therapeutic strategies for individuals with allergies.

Key words: Central goods and services tax, cross reactivity, gaint hazel, l-goods and services tax, pollinosis, wild pivet

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 that 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] Cora1 showed amino acid sequence identities[10] with seed storage proteins, *Sesamum indicum*[11,12] (Ses i 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 Cora1-like allergens, which could be used as analogous allergens and predictors of clinical sensitization. Immunoglobulin E (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 characterized well.[16] Goods and services tax (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

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population, and cross-reactive allergens have been found in ash, lilac, and *Ligustrum*.[18]

MATERIALS AND METHODS

Purification of GST from *L. robustum*

Flowers of *Ligustrum* were made into Smoothie[19] and GST purification from the extract was performed as per manufacturer instructions (Takara Bio). Eluted protein samples were pooled and filled into a dialysis membrane with a cutoff of 6–8 kDa (Pall Corporation). Protein suspension was dialyzed in approximately 2 L of dialysis buffer and the buffer was changed for 4 times against sodium phosphate (Na2HPO4, 50 mM, pH = 8.0) buffer to remove cell toxic imidazole.[20]

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (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 2× 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 (ThermoMultiskan). The absorbance was determined every 5 min over a time of 1 h.[23]

Determination of pollinosis by enzyme-linked immunosorbent assay (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 1 h at RT. 100 µL/well of purified immunoglobulin G (IgG) and or IgE (1:1000 in PBST; puregene) were added and incubated for 30 min at 37°C followed by 60 min at 4°C. The plates were washed 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 (ThermoMultiskan, 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 buffer overnight at 4°C or for 1 h at 37°C. Subsequently, the plates were washed twice with PBST andblocked with 150 µL/well of PBST along with 1% BSA for 2 h at RT. 100 µL/well of anti-human IgE and anti-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 (ThermoMultiskan, USA).[25]

RESULTS AND DISCUSSION

Purification of GST from *L. robustum*

Stained protein bands at a size of 32kDa represent GST produced in satisfactory yields. The pooled pre-purified protein sample was dialyzed and analyzed by SDS-PAGE. The gels were scanned on a Gel scanner with a white light converter (UVI-Tech, Lark Innovative) and the resulting image was analyzed with UVI-Tech Software and depicted in Figure 1.

Enzymatic activity of ligustrum GST

The enzymatic activity of GST and BPE was investigated in a GST activity assay kit. The results showed that Corylus GST 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 [Table 1] by

Table 1: Enzymatic activity of GST measured in GST activity assay kit and expressed in U/mL, respectively

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

*Bee pollen extract. GST: Goods and services tax

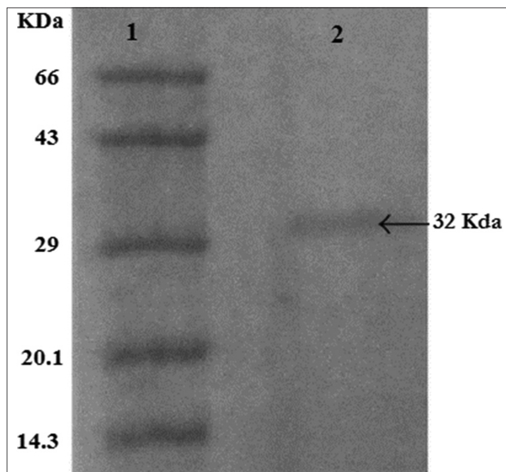

Figure 1: Analysis of central goods and services tax protein on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Lane 1 represents the protein marker (Genei) and Lane 2 represents the protein band at calculated size of 32KDa

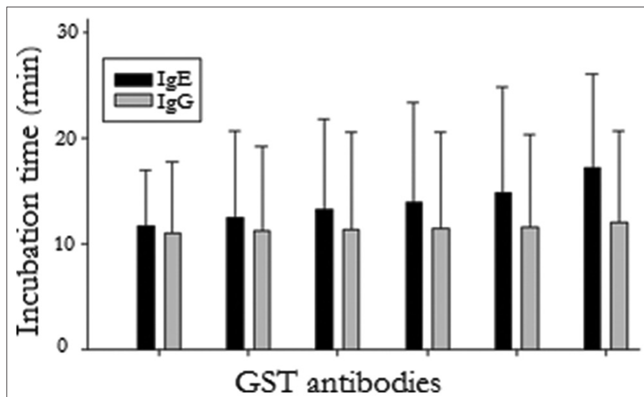

Figure 2: Central goods and services tax antibodies raised toward the VT20 and Cor a1 pollens at different intervals. n=3 for all groups, values are means ± SE

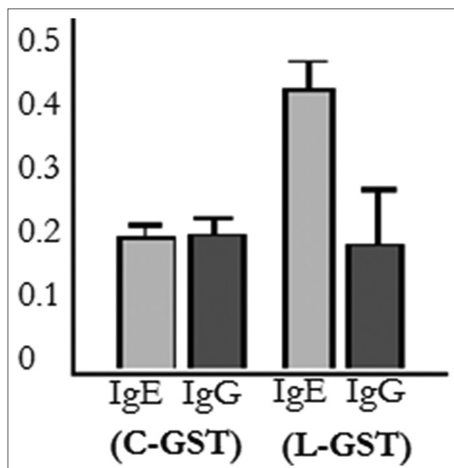

Figure 3: Detection of central goods and services tax (GST) specific antibodies cross-reacting with Ligustrum pollens, but not seen in Corylus GST

a suitable substrate BITC at 274 nm which is used for the broadest range of GST isozymes.

Determination of pollinosis by ELISA

VT20 pollen showed a specific IgG response and antibody titers increased after every regular intervals [Figure 2]. IgE antibodies level did not show a significant raise after incubation neither in the VT20 nor in the Cor a1 group. A moderate increase of IgE antibody titers 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.

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 [White bars in Figure 3]. In contrast, GST-specific IgG clearly recognized GST in VT20 [Grey bars IN Figure 3].

CONCLUSION

GSTs belong to a well-conserved enzyme superfamily which is, 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 attempted to identify the GST levels in *Ligustrum* and the cross-reactivity of antibodies toward 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, it will be elucidated in future experiments.

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