

# Chitin-Binding Mistletoe Lectin (MChbL) Exhibits Entomotoxic and Cytotoxic Properties

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## Abstract

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*Apamea sordens* Hufn. and *Agrotis segetum* Schiff. are serious herbivore Lepidoptera pests on various agricultural crops causing substantial crop losses throughout the world. They are responsible also for significant damage of stored seeds and post-harvest loss of agricultural production. Plant agglutinins (lectins) as natural plant defense agents, have been implicated as antibiosis factors against insects and are promising candidates for biological pesticides. The insecticidal activity of *Viscum album* chitin-binding lectin (MChbL) against *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. larvae was investigated. MChbL exhibited proteinase inhibitory and chitinase activities and affected larval development and survival at different growth stages. The rate of adults successfully emerging from pupae fed on MChbL was from 5% to 33%, when incorporated into an artificial diet at a level of 0.01% (w/w). MChbL decreased larval total midgut protease activity by 60% at a concentration of 0.25 µg/µl. Toxic properties of chitin-binding mistletoe lectin (MChbL) against Lepidoptera pests and human peripheral blood lymphocytes have been investigated. High concentrations of MChbL exhibit cytotoxic properties. Lectin was not cytotoxic to human peripheral blood cells at the concentrations of 10 µg/ml and exhibited similar results as ConA at sub-mitogenic concentration. In short term feeding trials MChbL did not retard animal mass growth and did not affect overall conditions of male or on female animal groups at the concentrations up to 0.1% (w/w). N-terminal amino acid sequencing of MChbL showed homology to plant pathogenesis-related (PR) protein families with 60% homology. MChbL could be useful in the development of entomotoxic biopesticides for the control of Lepidoptera pests at a dose-dependent manner.

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## Introduction

Insects are in competition with human agro-industry. Losses in agricultural production due to insect pests have been estimated at 15% of total production worldwide, especially in those countries which are the most dependent on agriculture for their subsistence or for the revenue it generates for health and development expenses. *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. are serious herbivore Lepidoptera pests on various agricultural crops causing substantial crop losses throughout the world. They are responsible also for significant damage of stored seeds and post-harvest loss of agricultural production. Intensive use of agrichemicals results in increased pest resistance and subsequently, intensification of pesticides applied to the fields which are toxic to humans and harmful to the environment. Due to the environmental concerns of pesticide use and limited list of effective alternatives it is therefore urgent to develop novel biological pesticides from plants and other natural sources that have low mammalian and environmental toxicity.

Lectins are among wide range of natural defense proteins found in plants (Rudiger & Gabius, 2001). They are heterogeneous group of proteins classified together on the basis of their ability to bind in a reversible way to well-defined simple sugars and/or complex carbohydrates. The main characteristic of these proteins is their ability to interact specifically with carbohydrates and to combine with glyco-components of the cell surface. While the physiological functions of plant lectins have not yet been fully elucidated, one possible function that of serving as a chemical defense against large array of insect pests is well documented (Carlini & Grossi-de-Sá, 2002; Vasconcelos & Oliveira, 2004). Plant lectins or phytoagglutinins are capable of recognizing and binding glycoconjugates on exoskeleton of insects or specific sites exposed along intestinal tract. Such interaction is considered to be prerequisite for insecticidal action. The insecticidal activity against a large array of insect species belong to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented (Coelho *et al.*, 2007; Ohizumi *et al.*, 2009). Insecticidal activities were found to be associated mostly with the two main groups of plant lectins: monocot mannose-binding and chitin-binding lectin groups. Snowdrop (*Galanthus nivalis*) bulb lectin was shown to be insecticidal to a range of economically important pests (Fitches *et al.*, 2001; Rudiger & Gabius, 2001). GNA and wheat germ agglutinin are increasingly used in development of transgenic crops (Stoger *et al.*, 1998).

European mistletoe (*Viscum album*) is a semi-parasitic plant. Mistletoe preparations have been used for pharmacological purposes mainly in conventional cancer therapy for decades (Tabiasco *et al.*, 2002; Gong *et al.*, 2007). There are indications that the therapeutic applications of this medicinal plant are linked to the biological activities of its lectins. Extracts from mistletoe are used in antitumoral treatments (Zarkovic *et al.*, 2001; Gabius *et al.*, 1996) based on direct toxicity of mistletoe lectins to tumor cells (Baxevanis *et al.*, 1998; Valentiner *et al.*, 2002). The *in vitro* administered mistletoe extracts and pure mistletoe lectins inhibited the growth of all tumor cell lines thus show the therapeutic effects (Jansen *et al.*, 1993). Therapeutic efficacy is mostly attributed to the mistletoe lectins (MLs), which belong to the “toxic lectin family” and represent type-2 ribosome-inactivating proteins (RIP) (Barbieri *et al.*, 1993). They consist of an N-glycosidase (A chain) and a galactosid-binding lectin (B chain) linked by a disulfide bridge.

Mistletoe is considered to be a toxic plant, and its content of toxic lectins lends support to this. Poison centers report toxicity of the whole plant, but especially the berries (Stirpe, 1983). Mistletoe proteins, particularly, viscotoxins and some mistletoe lectins known to be toxic to mammals, on tumor cell lines in culture (Urech *et al.*, 1995). Viscotoxin A3 belonging to antimicrobial plant peptides (AMPs) is a representative of thionins (Stotz *et al.*, 2013). The activities of plant AMPs are primarily directed against fungal, oomycete, and bacterial microorganisms, but certain members of a class can be directed against other targets, including herbivorous insects. In this report, the chitin-binding lectin from berries of *Viscum album* (MChbL) was checked by an insect bioassay for its insecticidal activity toward *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) larvae and determine values of the toxin via an insect bioassay with different

concentrations of MChbL. Also, we investigated the cytotoxicity of above lectin towards human peripheral blood lymphocytes (PBL) and determine its possible mammalian toxicity by monitoring overall conditions of an experimental animal *in vivo* in short term feeding trails.

### Methodology

The fruits of European mistletoe were harvested in mountainous region of East-Georgia, in winter (December-February) and stored at  $-15^{\circ}\text{C}$  until use. Mistletoe chitin-binding lectin (MChbL) was prepared as described with some modifications (Keburia & Alexidze, 2001). The plant material was homogenized in medium consisting of 0.05 M Na-acetate buffer, pH 4.5, at ratio 1:3 (g/ml). The extracts were centrifuged at  $5000 \times g$  for 15 min; supernatant was filtered through Miracloth (Calbiochem, USA) and Watman GF/c filter. The soluble protein fractions were purified by chromatography on the agarose (Serva) and chitin (Sigma) sorbents. Then the lectin was chromatographed on a chitin column (4-mL bedvolume) equilibrated with 50mM phosphate buffer, pH 7.6. After adsorption of the proteins (2 mg of MChbL in 200  $\mu\text{L}$  of phosphate buffer), the column was washed with the same buffer until the absorbance at 280 nm returned to zero, after which the adsorbed MChbL was eluted with 0.1 M acetic acid. Fractions were collected, and proteins were estimated based on the absorbance at 280 nm. Lectin was dialyzed, lyophilized and stored until use.

The larvae of *A. sordens* and *A. segetum* were obtained from Khashuri region (East Georgia). Larval cultures were reared continuously at  $25 \pm 1^{\circ}\text{C}$  and relative humidity of 65-75%, under a L16/D8 light regime. To examine the effects of MChbL on insect larvae, they were maintained in plastic boxes, with perforated plastic covers and reared on a control and experimental diet with or without lectin, respectively. The lectins were incorporated into natural diet daily at a level of 0.001% (w/w). 10-15 larvae were used per treatment. Insect survival was estimated daily, the weights of larvae and pupae were measured and the duration of developmental stages was determined. The effect of MChbL on the development was assessed by determining the number and mass of surviving larvae.

Total gut protease activity was measured by FITC-casein assay. Fluorescein isothiocyanate was purchased from Sigma Chemical Co (USA). FITC labeled casein was prepared as follows: casein (10 mg) and FITC (4 mg) were dissolved in 2 mL of 0.1 M sodium carbonate buffer (pH 9.0) containing 8 M urea and left for 3 h at  $20^{\circ}\text{C}$ . FITC labeled casein was separated by gel chromatography on a Sephadex G-25 column (10 mL) equilibrated with 10 mM phosphate buffered saline (pH 7.5) (PBS); the visible FITC-casein fraction was pooled, desalted by dialyzing against distilled water, and lyophilized.

Midguts were isolated by dissecting the fifth instar larvae. The gut tissue was mixed with 3 volumes of 0,1 M Gly-NaOH buffer (pH 10.0) and allowed to stand for 15 min on ice to extract proteases. The gut luminal contents were recovered by centrifugation at 10,000 g for 10 min at  $4^{\circ}\text{C}$ . The resulting supernatant was analyzed for protease assays. MChbL was preincubated with gut extract at  $37^{\circ}\text{C}$  for 15 min, prior to addition of the substrate. The enzyme solution (20  $\mu\text{L}$ ) was added to 40  $\mu\text{L}$

of FITC-casein (1  $\mu\text{g/ml}$ , in 0.1M Gly-NaOH buffer, pH10.0) and incubated at 37°C for 1 h. The reaction was stopped by adding 5  $\mu\text{l}$  of 60 % trichloroacetic acid (TCA). The solution was mixed with 200  $\mu\text{l}$  of 0.2 M Tris-HCl buffer (pH 9.0) containing 0.5% SDS and 0.02%  $\text{NaN}_3$ . The fluorescence polarization of samples was measured with Ex: 490 nm and Em: 520 nm. Each assay was carried out in triplicate. Trypsin Inhibitor Activity was determined by a continuous rate spectrophotometric assay and expressed as the inhibition of BAEE units. Soybean trypsin inhibitor from *Glycine max* (soybean) was used as standard.

Male and female mice lines were reared in the Medical University animal house (Tbilisi, Georgia) on an artificial diet containing MChbL at concentrations of 0.001%, 0.05%, and 0.1% (w/w) or equivalent amount of bovine serum albumin (BSA, Sigma) as a control diet. Each group of experimental animals composed of five male and five female individuals having average weight 18-22 g of each. Each treatment was administered as 0.5 ml liquid supplement prior feed and was replicated after a 24-h period. Five groups were used per treatment. Fifth group was considered as blank and contained no lectin or BSA in artificial diet. Monitoring was accomplished twice per day. Mice survival and overall conditions was estimated daily, and the weights of individuals were measured. Trypsin inhibitor activity was determined by a continuous rate spectrophotometric assay and expressed as the inhibition of BAEE units. Soybean Trypsin Inhibitor from *Glycine Max* (soybean) was used as standard.

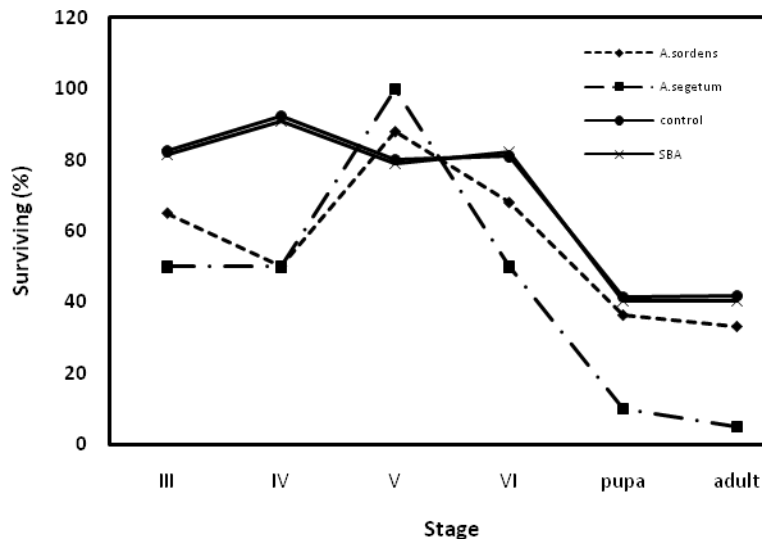
Human peripheral blood lymphocyte (PBL) culture was used to study MChbL cytotoxicity by mitogen stimulated dimethylthiazol, diphenyltetrazolium bromide (MTT) assay. Separation of peripheral blood lymphocytes (PBL) was performed as follows: The peripheral blood of 10 healthy donors aged 20-50 were studied. Each sample of whole blood was diluted 1:1 ratio with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  free Hank's balanced salt solution (HBSS, Gibco) and 10 ml of this mixed blood was overlaid onto 3 ml Histopaque (1.077  $\text{g/cm}^3$  density) solution (Sigma). After centrifugation at  $770 \times g$  for 45 min at room temperature, the interphases of PBL was aspirated and cell suspension was washed twice in HBSS at  $400 \times g$  for 10 min, re-suspended in 1 ml of medium RPMI 1640 (Sigma), counted in Haemocytometer and concentration was adjusted at  $2 \times 10^6$  ml with medium, supplemented with 10% fetal bovine serum (FBS, Sigma). 100  $\mu\text{l}$  of this suspension was added into wells of 96 well microplate in duplicates and each well was filled with 80  $\mu\text{l}$  media supplemented with 20  $\mu\text{l}$  mitogen (ConA, Sigma) or 20  $\mu\text{l}$  MChbL. The wells without mitogen or MChbL were considered as blank (B1) wells. Different dilutions of MChbL protein and mitogen were used. 20  $\mu\text{l}$  of MTT solution (Sigma, 5 mg/ml PBS - phosphate buffer solution) was added into each well after 72 h incubation time at 37°C. During next 4 h incubation time the formazan crystals were produced. The media was removed from wells carefully and 100  $\mu\text{l}$  solution of 10% SDS (sodium lauryl sulfate), 0.1M HCl was added. After incubation at 37°C 3 h the crystals were dissolved and the optical densities were estimated based on the absorbance at 570 nm using spectrophotometer Multiscan MCC.

All data were examined using one way analysis of variance (ANOVA). The student test was used to identify the means which differed when ANOVA test indicated significance. A  $p$  value  $< 0.05$  was considered to be significant (IBM SPSS Statistics).

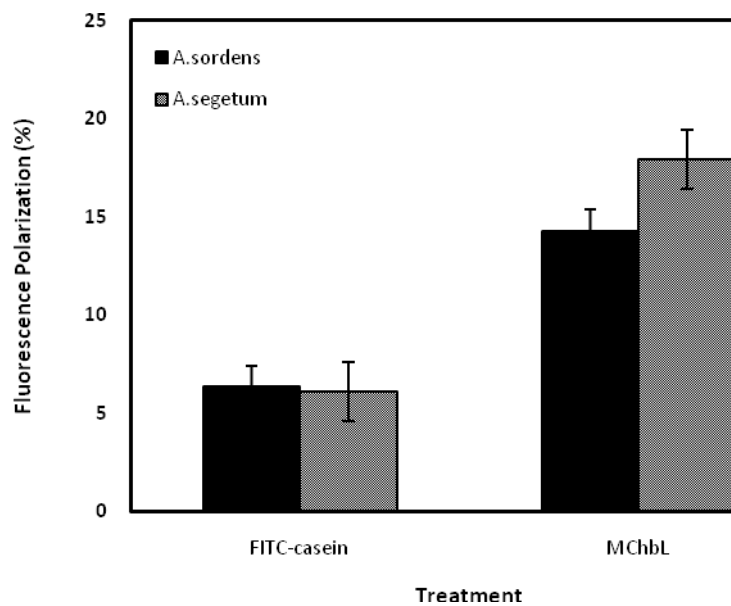
### Results and Discussion

The effect of MChbL on the larvae development was assessed by determining the number of surviving larvae. Larval mortality in experimental groups was higher than that of control groups containing BSA as supplement to an artificial diet or without any additives. Third instar larvae were reared on an artificial diet containing MChbL and SBA at a concentration of 0.001%. In a bioassay from third instar to adult, the rates of adults successfully emerging from pupae fed on MChbL were 33% and 5%, respectively (Figure 1). These rates were much lower especially concerning to *A. segetum* than those of control insects (41.7%). The survival of third instar larvae of *A. sordens* and *A. segetum* fed on MChbL were 65% and 50%, respectively, compare to that of control insects (82%). The results showed that the influence of lectins were much evident at the early stages of larval development. Apparently, this may be related to glycosylation degree of the gut structures of newly emerging insects. In contrary, at the following stages (from third to fifth instar) of development MChbL did not show significant influence on *A. sordens* larvae survival. Accordingly, *A. sordens* larvae showed to be less susceptible to deleterious effects of lectins at their late developmental stages in compare to *A. segedum*.

The inhibitory effects of MChbL on midgut proteases activity measured by FITC-casein assay is shown on Figure 2. Proteolytic activity of the midgut extracts from fifth instar larvae was measured by fluorescence polarization spectroscopy using FITC-labeled casein as substrate. The results showed that MChbL influenced larval gut proteolytic enzymes activity (decrease of total protease activity of the midgut extracts was monitored). The highest inhibition was 60% at a concentration of 0.25  $\mu\text{g}/\mu\text{l}$  MChbL. When incubated with the insect enzymes MChbL showed resistance to digestion and no inhibition of sugar-binding activity of lectin was observed. Resistance to degradation by pest metabolic systems is clearly beneficial for plant defensive proteins, production of which represents an effective strategy developed by some plants (Brunelle *et al.*, 2004).



**Figure 1:** Effect of MChbL on the survival and development of *A.sordens* and *A.segetum* when incorporated into an artificial diet at 0.001% (w/w). Insects were newly emerged third instars larvae at the start of the assay.



**Figure 2:** Effect of the MChbL on the proteolytic activity of midgut extracts from *A.sordens* and *A.segetum*. FITC-casein: substrate solution of FITC-casein was added to *A.sordens* and *A.segetum* midgut extracts; MChbL: *A.sordens* and *A.segetum* midgut extracts were preincubated with MChbL (ANOVA, n=20, p<0.05; Student's T-Test).

The effect of MChbL on the overall conditions of experimental animals was assessed by monitoring of the mass of surviving animals fed a diet containing increasing amounts of MChbL. The applied doses to first three groups were 0.25 mg/kg, 12.5 mg/kg, and 25 mg/kg respectively, which corresponded to MChbL doses of 0.001%, 0.05% and 0.1% (w/w) respectively. The minimal concentration of MChbL (0.001%) was selected according to that negatively affected *A. sordens* and *A. segetum* larval development and survival at different growth stages.

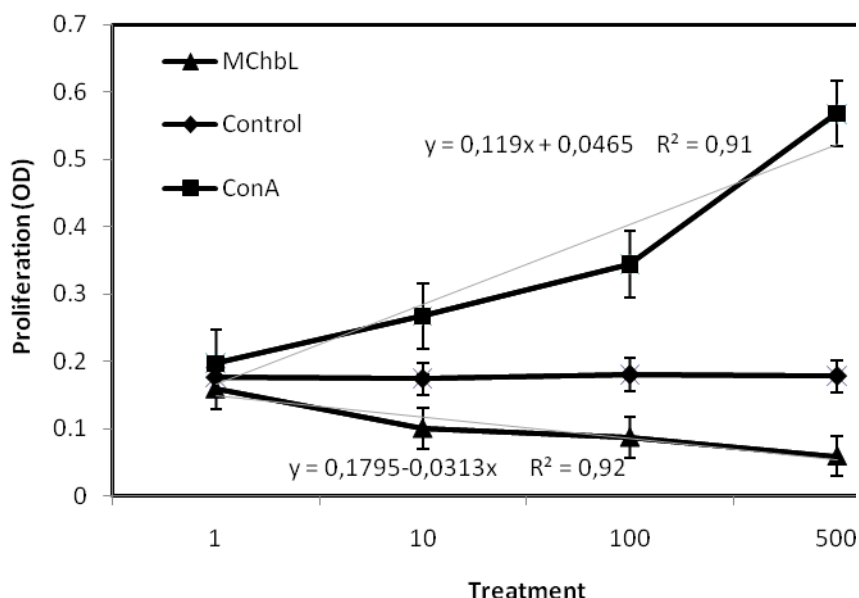
The dose response effect of MChbL on the weight growth of the animal groups by days is shown in Table 1. Apparently, no considerable reductions in animal weights were observed during short term feeding trails. Overall conditions were monitored twice a day. No changes in overall conditions were noticed among male or female animal groups in compare that of control ones including characteristics such as behavioral changes, movement intensity and coordination, tonnnus of muscles, response on external irritants were monitored. Observations were made also at breath frequency, developments of convulsion, positions of fleece and tail. The variations in mice weight changes did not exceed admissible 10% limit. Statistical analysis revealed that doses of MChbL applied to experimental animals ( 0.001% to 0.1% ; w/w) did not affect animal mass therefore had no obvious toxic effects neither on male nor on female animal groups. Statistical analysis has revealed the significant differences ( $p<0.01$ ) in weights at the beginning and days of feeding experiments between male and female animal groups, while survival and weight deviations of control groups and experimental animals did not yield valuable difference ( $p<0.5$ ). These data point out the advanced growth of experimental animal groups likewise control ones.

**Table 1:** The dose response effect of MChbL on the weight growth (mg) by days of the male (A) and female (B) animal groups<sup>a</sup>.

	1	2	3	4	5	6	7	8	9
<b>(A) males</b>									
control	24.5±1.06	25.1±0.71	25.4±0.35	25.1±1.13	25.6±0.71	25.5±0.92	25.6±0.28	25.9±0.50	25.4±0.28
BSA	23.8±1.84	24.1±1.34	23.8±1.91	23.5±1.70	23.0±0.99	22.5±1.06	22.2±0.28	22.1±0.42	21.7±0.99
Group I	24.2±0.25	24.1±1.21	25.3±0.48	25.6±0.92	25.5±0.68	25.5±0.98	25.2±0.95	25.4±0.78	25.4±0.91
Group II	22.1±0.47	23.0±1.06	23.7±0.84	22.1±1.22	22.3±1.27	21.6±1.82	22.2±1.87	22.9±1.91	23.0±1.56
Group III	21.0±0.51	21.7±0.32	21.7±0.95	21.7±0.85	22.7±0.21	22.7±0.28	23.3±0.21	23.5±0.28	23.4±0.28
<b>(B) females</b>									
control	16.6±1.70	16.9±2.03	17.2±1.77	17.3±1.91	17.6±2.19	17.3±2.12	17.8±1.56	18.8±2.05	18.4±1.34
BSA	17.8±0.30	18.1±0.14	17.9±0.10	16.6±0.85	17.1±0.14	17.0±0.35	17.2±0.14	18.2±0.28	18.8±0.21
Group I	18.3±2.02	19.3±1.75	20.4±1.96	20.5±1.52	20.3±1.31	20.8±1.61	21.2±1.40	20.9±0.93	21.0±1.18
Group II	18.4±0.68	18.3±0.93	19.5±0.92	19.7±0.44	20.0±0.17	20.0±0.20	20.2±0.55	19.9±0.75	20.3±0.87
Group III	17.8±0.75	18.3±0.57	19.6±0.41	19.1±0.98	19.1±1.04	19.3±0.85	19.2±1.2	18.4±1.12	20.1±0.75

<sup>a</sup>Values are means ±std. deviation, n=5.

Trypsin inhibitor activity of MChbL was conducted using soybean trypsin inhibitor from *Glycine Max* (soybean) to estimate influence of MChbL on gut proteolytic activity in mammals. MChbL showed no trypsin inhibitory activity towards bovine trypsin (data not shown), indicating the possible digestibility of MChbL by mammalian gut enzymes.



**Figure 3:** Proliferation of human peripheral blood cells. Human peripheral blood lymphocyte (PBL) were supplemented with different concentrations of mitogen (Con A) or MChbL. Control indicates the wells without mitogen or MChbL. Bars indicate mean  $\pm$  Std. deviation; (ANOVA,  $n=10$ ,  $p<0.05$ ; Student's T-Test).

Human peripheral blood lymphocyte (PBL) culture was used to study MChbL cytotoxicity by MTT assay. The MTT assay is colorimetric assay for measuring the activity of enzymes that reduce yellow MTT to purple formazan crystals, in the metabolically active mitochondria of living cells. The main application allows assessing the viability and the proliferation of cells. It can also be used to determine cytotoxicity of potential different agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth. The reduction of tetrazolium salts is now recognized as a safe, accurate alternative to radiometric testing (Wilson, 2000). The formazan crystals are solubilized by the addition of a detergent. The color can then be quantified by spectrophotometric means, measuring at a certain wavelength (usually between 500 nm and 600 nm). The reduction takes place only when mitochondrial reductase enzymes are active and therefore conversion can be directly related to the number of live cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated cells, the effectiveness of the agent in causing death of the cells can be deduced. Increasing cell number result correlates with an increase amount of MTT formazan formed and an increase in absorbance. The result of study is shown in Figure 3. Any of doze of mitogen (Con A) have stimulated the human peripheral blood lymphocytes OD – 0.344 (100 $\mu$ g), 0.467 (10 $\mu$ g) and 0.198 (1 $\mu$ g), in compare to blank control OD 0.173, whereas MChbL applied at concentrations of 10  $\mu$ g/ml and 500  $\mu$ g/ml slightly inhibited the proliferation of PBL cells (OD 0.100 and OD 0.060 respectively). Decrease in amount of the viable cells was much obvious at high concentration of MChbL applied (OD 0.060).

MChbL demonstrated a significant antiproliferative effect on PBL when administrated at high concentration. Apparently, high doses of MChbL negatively affects cell functioning and accordingly, exhibit cytotoxic properties. In contrary, low concentrations of MChbL were less cytotoxic to



peripheral blood cells and exhibit similar results as Con A at the concentration of 1 µg/ml. Some plant lectins expose toxic and pathological lesions on mammals. Mistletoe preparations are given in incrementally increasing dosages depending on the patient's general condition and response to the injection (Horneber *et.al.*, 2008; Thies *et.al.*, 2008). Most likely, approaches to using MChbL preparation must be considered in dose-dependent manner in nontoxic range.

The N-terminal domains of MChbL were determined by applying of carboxamido-methylated proteins to sequential Edman degradation. The first 20 amino acids in N-terminal region of MChbL 23 kDa polypeptides showed the following sequence: Asp-Glu-Pro-Val-Val-Arg-Asp-Gln-Ala-Pro-Asp-Thr-Leu-Trp-Ala-Ala-Ala-Lys-Pro-His. Homologous sequences searched by FASTA program revealed high homology between NH<sub>2</sub>-terminal domains of MChbL and deduced amino acid sequences of thaumatin protein family: osmotin-like protein from *Heveabrasiliensis* and α-amylase/trypsin inhibitor from *Zeamays* with 60% homology, thaumatin-like protein from *Actinidiachinensis* (Kiwi plant) with 61.5% homology and pathogenesis-related protein from *Juniperusvirginiana* with 53.3% homology (Figure 4). In contrary, no sequence homologies were found between MChbL and plant toxins like RIPs or other mistletoe chitin-binding ViscalbcBA and cbML lectins. Thaumatin-like proteins belong to pathogenesis-related (PR) protein group expressed in the plants upon elicitor induction. Despite the structural divergence with thionins and hevein-containing other chitinases, high homology with α-amylase/trypsin inhibitor from *Zeamays* might be the prerequisite for the antinutritive effects of MChbL, thus contributing defense against herbivorous pests.

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TLP_ACTCH  -----*ATFNFINNCPFTVWAAAVP-G----- 20
IAAT_MAIZE -----*AVFTVVNQCPFTVWAASVPVGGGRQLNRGESWRITAPAGTT 41
OLPA_HEVBR -----*ATFTIRNXPYTVWAAASP-GGRRLD----- 26
MChbL      -----*DEPVVRNQAPDTLWAAALPH----- 20

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**Figure 4:** Aligned amino acid sequences of NH<sub>2</sub>-terminal domains of MChbL and homologous proteins. MChbL, chitin-binding lectin from *V. album*; TLP\_ACTCH, thaumatin-like protein from *Actinidia chinensis*; IAAT\_MAIZE, alfa-amylase/trypsin inhibitor from *Zea mays*; OLPA\_HEVBR, osmotin-like protein from *Hevea brasilienses*; PRR3\_JUNVI, pathogenesis-related protein (PR) from *Juniperus virginiana*. The the conserved amino acid residues in NH<sub>2</sub>-terminal domains are indicated by asterisks.

The results obtained demonstrate that mistletoe chitin-binding lectin has obvious anti-nutritive effects on Lepidoptera larvae. Apparently, lectin exerts its toxicity at early stages of development by interaction with midgut structures. The precise mechanism how the lectin exerts the insecticidal activity has not been fully elucidated. However, since glycoproteins are the major constituents of insect gut structures, it is possible that specific interaction take place between the

glycosylated gut structures and plant lectins (Zhu-Salzman *et al.*, 1998). It appears, that surviving the hostile proteolytic environment of the insect midgut, specific binding to insect gut chitin components and alteration of glycosylated enzymes of digestive tract are basic prerequisites for MChbL lectin to exert its deleterious effects on insects. The insecticidal activity of defense against herbivorous pests may be attributed to the lectin-induced reduction in diet ingestion resulting starvation of larvae.

Due to its high sensitivity characteristic, FTIR spectra can reflect small variations due to culture parameters such as type of media used, temperature, storage mode and age of culture. Therefore, a standardized preparation procedure should be taken into consideration to achieve a high level of spectrum reproducibility that is crucial to avoid misidentification (Santos *et al.*, 2010).

## Conclusion

Controlling insects generally requires the use of chemical insecticides which are toxic to humans and domestic animals and harmful to the environment. Chitin-binding lectin (MChbL) from mistletoe berries showed antinutritive properties towards herbivore Lepidoptera pests *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae) by affecting the larval development and survival at different growth stages. Homology to thaumatin protein family, best known as  $\alpha$ -amylase/trypsin inhibitors and pathogenesis-related (PR) protein groups suggests the possible protection function of MChbL against Lepidoptera pests. Low cytotoxicity of MChbL at entomotoxic concentrations towards peripheral blood lymphocytes and non-toxic effects on mammals *in vivo* tempt to be speculated as natural biopesticide for the control of polyphagous herbivore pests in dose-defend manner.

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