Malaysian Journal of Science **26** (2): 41 – 46 (2007)

Hypoglycemic, Insulinotrophic and Cytotoxic Activity of three species of *Ganoderma*

Mohd. Saufi Bastami[1], Siti Pauliena Mohd Bohari[1], Wong Mee Har[1], Mohd. Noor Wahab[1], Ariffi Suraya Rahmani[2], Nor Fadilah Rajab[2] and Muhajir Hamid[1]*

[1] Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Selangor, Malaysia
* muhajir@biotech.upm.edu.my, + 6 03 89466707 (telephone), + 6 03 89430913 (facsimile) (corresponding author)
[2] Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Aziz, 50300 Kuala Lumpur, Malaysia
Received 8th January 2007, accepted in revised form 11th September 2007.

ABSTRACT The crude extracts of *Ganoderma lucidum*, *Ganoderma tropicum* and *Ganoderma tsugae* were studied for insulinotrophic and hypoglycemic activities using rat pancreatic β-cell lines, BRIN-BD11 cells and normal Wistar rats. In an oral glucose tolerance test (OGTT), oral administration of 1 g/kg body weight of the crude extract resulted in a significant reduction in blood glucose in the normal rats. In acute 30 min insulin secretion tests, 1 mg/ml crude extract of all tested *Ganoderma* evoked a 1 - 4 fold stimulation of insulin secretion from the pancreatic β-cell lines. Of these, *G. lucidum* showed the highest insulinotrophic activity. In addition, cytotoxicity assay of extracts from *G. lucidum*, *G. tropicum* and *G. tsugae* towards rat pancreatic β-cell lines showed half maximal inhibition (IC_{50}) at 200, 100 and 150μg/ml, respectively. These results demonstrated the presence of insulin-releasing and hypoglycemic activity in the crude extracts of *G. lucidum*, *G. tropicum* and *G. tsugae*.

ABSTRAK Kajian terhadap aktiviti insulinotrofik dan hipoglisemik ekstrak kasar *G. lucidum, G. tropicum* dan *G. tsugae* telah dijalankan ke atas sel selanjar pankreas β tikus, BR1N-BD11 dan tikus normal Wistar. Dalam ujian toleransi glukosa oral (OGTT), administrasi ekstrak kasar sebanyak 1g/kg berat badan tikus secara oral didapati telah mengurangkan kandungan glukosa darah secara signifikan pada tikus normal. Dalam kajian perembesan insulin akut selama 30 min, kesemua jenis ekstrak kasar *Ganoderma* pada 1mg/ml meransang antara 1 - 4 kali ganda perembesan insulin daripada sel selanjar pankreas β. *G. lucidum* telah menunjukkan aktiviti insulinotrofik tertinggi. Selain itu, asai sitotoksisiti bagi ekstrak daripada *G.lucidum, G. tropicum* dan *G. tsugae* ke atas sel pankreas β tikus menunjukkan nilai separuh perencatan maksima (IC_{50}) masing-masing pada 200, 100 dan 150ug/ml. Hasil daripada kajian ini menunjukkan kehadiran aktiviti perembesan insulin dan hipoglisemik dalam ekstrak kasar *G.lucidum, G. tropicum* dan *G. tsugae*.

(*Ganoderma*, hypoglycemic, insulinotrophic, rat pancreatic β-cell lines)

INTRODUCTION

Diabetes has become one of the main life-threatening diseases. According to World Health Organizations, the prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 200 and 4.4% in 2030 [1]. In both the developed and developing nations, the cases of diabetes are increasing. In 2003, 194 million people worldwide ranging in age from 20 to 79 years had diabetes. By 2025, this number is projected to increase by 72% to 333 million [2].

Ganoderma belongs to the Basidiomycetes class of fungi. Traditionally, it has been highly regarded as herbal medicine, claimed to alleviate or cure virtually all diseases [3]. It is one of the most widely researched natural remedies in Asia and in Chinese folklore the fruiting body of *Ganoderma* (Lingzhi) has been regarded as a

panacea for all types of diseases. These include hepatopathy, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leucopenia, diabetes, anorexia, mushroom poisoning, and debility due to prolonged illness [4]. The anti-diabetic studies especially on hypoglycemic and insulinotrophic activities have been concentrated mainly on *G. lucidum* but no scientific investigation was reported on the other species of *Ganoderma*. To our knowledge, no reports are available on the hypoglycemic and insulinotrophic activities of *G. tropicum* and *G. tsugae*.

Hence, the present study was undertaken to evaluate the insulinotrophic and hypoglycemic activities of the crude extracts of locally grown *G. lucidum*, *G. tropicum* and *G. tsugae*.

MATERIALS AND METHODS

Extraction of Ganoderma samples

Ganoderma lucidum, *Ganoderma tropicum* and *Ganoderma tsugae* were originally obtained from the Botany Department, National Taiwan University and were maintained at Laboratory of Mycology, Department of Microbiology, Universiti Putra Malaysia. All *Ganoderma* species were confirmed botanically and grown at 25°C. The fruiting bodies were harvested after three months of cultivation. Fruiting bodies were then cut into small pieces and dried in an oven at 45°C for 76 hrs. The dried fruiting bodies were subjected to a size reduction to powder by using a dry grinder and hot water soluble extract was formed by reflux distillation and this crude extract later was used for experiment.

Experimental animals

Male albino Wistar rats (body weight 150 – 250g) bred in the animal house in Faculty of Veterinary Medicine, Universiti Putra Malaysia were used in this study. The rats were divided into five groups (seven in each group) and maintained in ventilated animal room with free access to tap water and standard pellet diet.

Oral Glucose Tolerance Test (OGTT) in normal rats

Crude extracts of *G. lucidum, G. tropicum and G. tsugae* (500 and 1000 mg/kg body weight) were administered orally by using a canulla fitted needle attached to a syringe to experimental rats fasted for 15 hours. Distilled water was used as negative control and glibenclamide (5 mg/kg body weight) was chosen for standard drug control. Initial blood samplings were taken 30 minutes prior to oral administration of the extracts. After which, at t = 0, blood samples were taken and each group was given a glucose load of 1.5 g/kg body weight. Determination of blood glucose was then carried out at 30, 60, 90, 120 and 150 minute after the glucose loading. The blood was obtained by puncturing the tip of the tail with a lancet and the glucose level was determined using Acutrend® (Roche).

Insulin secretion in vitro

Insulin secretion was evaluated using BRIN-BD11 cell lines produced by electrofusion of immortal RINm5F cells with New England Deaconess Hospital rat pancreatic β-cells [5]. Secretory characteristics of these cell lines had been described intensively and widely used as *in vitro* model for insulin secretory studies [6, 7]. Cells were seeded at concentration of 2.5 X 10^5cells/well in a 24 well plate. The cells was then cultured in RPMI 1640 containing 11.1mmol/L of glucose supplemented with 10% (v/v) of foetal bovine serum and antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin) to allow attachment overnight prior to acute tests. Cells were washed thrice with Krebs Ringer Bicarbonate buffer (KRBB, pH 7.4) supplemented with 0.5% (w/v) bovine serum albumin and 1.1 mmol/L of glucose before preincubation for 40 minutes at 37°C. The cells were then incubated for 30 minutes with 1 mL KRB at 1.1 mmol/L of glucose in the absence and presence of extract. Following incubation, aliquots were removed from each well and stored at -20°C for insulin assay using ELISA method.

Insulin assay

The insulin level produced by BRIN-BD11 cells was measured by an enzyme-linked immunosorbent assay using a commercial rat insulin ELISA (DRG Instruments GmbH, Germany) and rat insulin was used as a standard [8]. It was based on the direct sandwich technique in which two monoclonal antibodies were directed against separate antigenic determinants on insulin molecule. During incubation, insulin in the sample reacted with anti-insulin antibodies bound to the microtiter wells and peroxidase conjugated anti-insulin antibodies. A simple washing step removed unbound enzyme labelled antibody. The bound

conjugate was detected by reacting with 3,3'5,5,'-tetramethylbenzidine (TMB) and read with the BioRad Reader at 450nm (reference filter 650nm).

Cytotoxicity assay

The cytotoxic effects of the extract on pancreatic β-cell line were determined using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay [9]. Cells were seeded in a 96-well plate at a concentration of 1 X 10^5 cells per well. The plate was incubated for 24 h at 37°C in an atmosphere containing 95% air and 5% CO_2 which allowed the cells to attach to the bottom of the wells. After which, the cells were treated at different concentrations of autoclaved *Ganoderma* extract for 72 hour. A volume of 20µl of MTT solution (5 mg/mL) was added to each well and incubated for 4 hour at 37°C. The medium was aspirated from the wells and 200 µL of dimethyl sulfoxide (DMSO) was added into each well to solubilize formazan. The plate was agitated on a plate shaker for 5 min and absorbance was measured at 570 nm with ELISA Reader. Dose-response curves were computer plotted after converting the mean data values to percentages of the control response. The half maximal inhibition concentration (IC_{50}) was evaluated from the dose-response curve.

Statistical analysis

All the data were statistically evaluated and the significance of various treatments was calculated using Students's unpaired t-test. Groups were considered to be significantly different if $P < 0.05$.

RESULTS

Effect on Glucose Tolerance on Normal Rats

The effect of a single dose of *Ganoderma* extract on OGTT is as shown in Figure 1. The extracts definitely resulted in lower blood glucose peak values 30 minutes after the glucose load compared to the negative control. Treatment with glibenclamide, *G. lucidum*, *G. tropicum*, and *G. tsugae* reduced the blood glucose levels to 67% ($P < 0.05$), 73% ($P < 0.05$), 72% ($P < 0.05$), and 75% ($P < 0.05$) respectively.

At 150 minutes after the oral administration of glucose, the blood glucose levels for glibenclamide, *G. lucidum*, *G. tropicum*, and *G. tsugae* further decreased to 52%, 56%, 65%, and 64%, respectively as compared to the negative control with ($P < 0.01$). All *Ganoderma* species showed no significant difference and exhibited almost similar pattern of blood glucose reduction. The results indicated that the crude extracts of the three species of *Ganoderma* reduced the glucose level in normal rats considerably.

Insulin Release Activity

Insulin secretion studies were performed to investigate the possible effects of the crude extract of *G. lucidum*, *G. tropicum*, and *G. tsugae* on *in vitro* insulin release activity. Figure 2 depicts the insulin-secreting activity of pancreatic β-cell lines after 30 minutes incubation with the extracts. Crude extracts of the three species of *Ganoderma* (0.1 - 2.0 mg/ml) had a dose-dependent stimulatory effect on insulin secretion by BRIN-D11 cells. Among the three *Ganoderma* species tested, *G. lucidum* showed the ability to stimulate insulin release in a stepwise pattern. The insulin secretion was decreased when BRIN-BD11 cells were treated with extracts of *G. tropicum* and *G. tsugae* with concentrations above 0.5 mg/ml. In this case, the decrease in insulin release was probably due to the cytotoxicity or inhibitory effect of the extract at higher concentrations. In order to determine whether cytotoxicity of the extracts affected insulinotrophic activity, IC_{50} value of three species of *Ganoderma* extract were examined. The respective IC_{50} values are as shown in Table 1. The cytotoxicity tests indicated that the IC_{50} values for *G. lucidum*, *G. tropicum*, and *G. tsugae* were 200, 100 and 145µg/ml, respectively. Generally, the results showed that cytotoxicity had an impact on insulin- stimulatory response. *Ganoderma* extract with low cytotoxicity level had higher insulin secretion activity, particularly extract from *G. lucidum*.

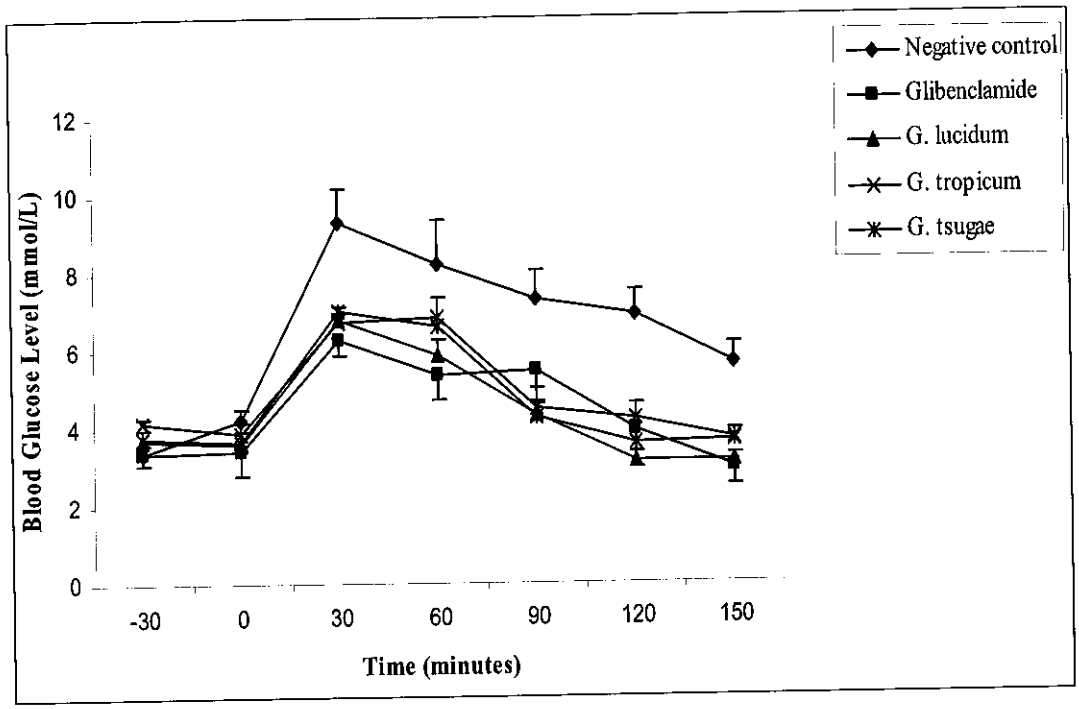

Figure 1. Oral glucose tolerance test in normal rats administered with *Ganoderma* extract (1 g/kg b.w.) and glibenclamide (5mg/kg b.w.).Values are expressed as means of blood glucose level for groups of 7 observations with their standard errors indicated in vertical bars.

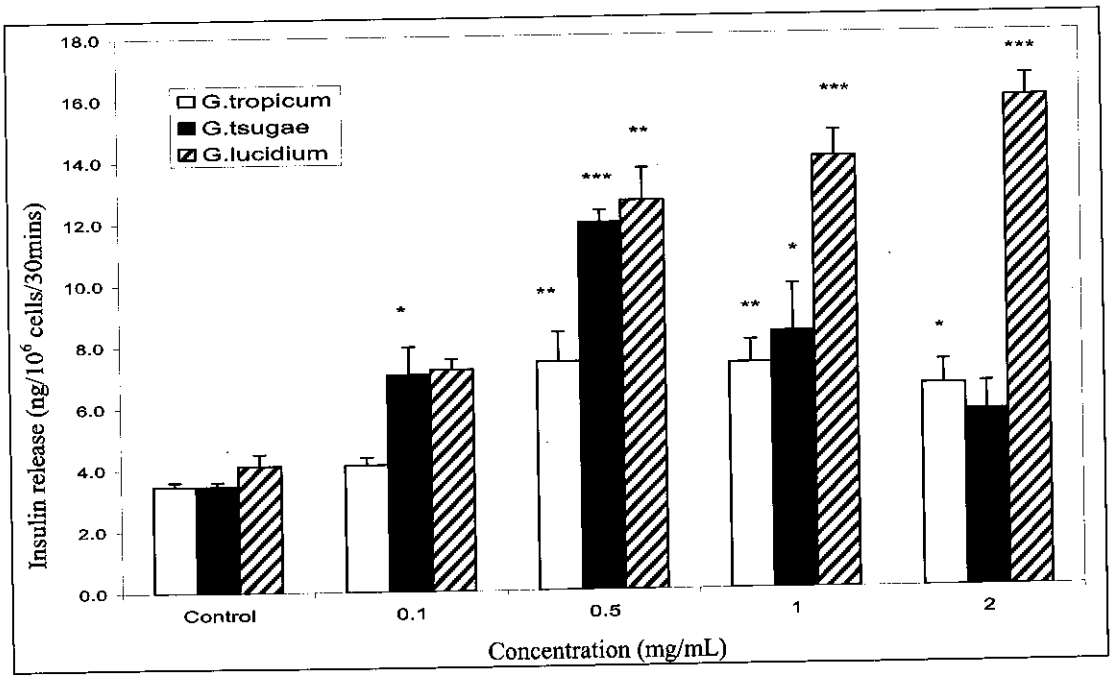

Figure 2. Effects of Ganoderma extract on insulin release by BRIN-BD11 cells. After 40 minutes of pre-incubation, insulin release was tested during 30 min incubation period. Values are mean ± SEM (n = 6). * P <0.05, ** P< 0.01, *** P <0.001 compared with control.

Table 1. Determination of half-maximal concentration (IC_{50}) of Ganoderma extracts inhibiting 50% of the BRIN-BD11 cells growth

SAMPLE	IC_{50} VALUE (μg/mL)
Ganoderma lucidium	200
Ganoderma tropicum	100
Ganoderma tsugae	145

DISCUSSION

The results of the hypoglycemic study showed that the crude extracts of *G. lucidum*, *G. tropicum* and *G. tsugae* had a hypoglycemic effect in normal rats. This could probably due to the presence of active hypoglycemic compound in extracts of all three species of *Ganoderma*. This is in agreement with the earlier findings by Kimura *et al.* [10] who suggested that the water extract of *G. lucidum* was found to reduce the blood glucose level in rats by the OGTT test.

In normal rats, hypoglycemic action of the crude extracts of *Ganoderma* species tested might be the result of the stimulatory effect of insulin secretion as shown in the present experiment. The ability of the three *Ganoderma* species to stimulate insulin secretion provided a good alternative enhancer in insulin secretion for diabetic patient with type 2 diabetes. Previous study by Hikino *et al.* [11] had reported that ganoderan B was the major hypoglycemic glycan isolated from *G. lucidum* and it enhanced glucose utilization by elevating the plasma insulin level. In recent years, it had also been demonstrated that *G. lucidium* polysaccrharides (GI - PS) had an effect on glucose reduction in normal fasted mice. Furthermore, they were able to protect pancreatic islets from free radicals-damage induced by alloxan [12, 13]. Although a lot of studies have been done on *G. lucidium* in terms of hypoglycemic activity, the two other species of *Ganoderma* namely *G. tropicum* and *G. tsugae* had also shown great potential in reducing blood glucose levels. To date, very little scientific investigation on anti-diabetic properties have been done on these two species. Similarly, very few studies have been carried out in the area of immunomodulatory and anticancer properties especially for *G. tsugae* [14, 15].

In conclusion, all the three *Ganoderma* species could probably contain some active components capable of stimulating insulin secretion giving rise to hypoglycaemic effect. Development of novel and sensitive techniques to detect, isolate, purify, and structurally characterize these biological active constituents might be useful for identification of specific active compounds produced by these *Ganoderma* species. Although investigation is still at its infancy stage, undoubtedly *Ganoderma* has the potential not only as a possible dietary adjunct for the treatment of diabetes but a potential source for the discovery of new orally active agents for future diabetes therapy.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial assistance provided by the Ministry of Science, Technology and Environment of Malaysia (Project No: 09 02 04 0328 EA 001), laboratory facilities of Universiti Putra Malaysia and Professor Peter R. Flatt, University of Ulster, U.K for providing the clonal of BRIN-BD11 cells.

REFERENCES

1. Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: Estimates for the year 200 and projections for 2030. *Diabetes Care* **27** (5): 1047 - 1153.
2. Narayan, K. M. (2005). The diabetes pandemic: Looking for the silver lining. *Clinical Diabetes* **23**: 51 – 52.
3. Chen, J. J. and Sih, N. L. (2002). Myth of Lingzhi, the mushroom of immortality: A potential Antitumor/Antiaging Agent in Humans. *Acta Cardiologica Sinica* **18**: 113 - 114.
4. Jong, S. C. and Birmingham, J. M (1992). Medicinal benefits of the mushroom *Ganoderma. Advances in Applied Microbiology* **37**: 101 - 134.
5. McClenaghan, N. H., Gray, A. M., Barnett, C. R. and Flatt, P. R. (1996). Hexose recognition by insulin-secreting BRIN-BD11 cells. *Biochemical and Biophysical Research Communications* **223**; 724 - 728.
6. McClenaghan, N. H., Elsner, M., Tiedge, M. and Lenzen, S. (1998). Molecular characterization of glucose-sensing mechanism in clonal insulin secreting BRIN-BD11 cell line. *Biochemical and Biophysical Research Communications* **242**: 262 – 266.

7. Welters, H. J., McBain, S. C., Tadayyon, M. Scarpello, J. H., Smith, S. A and Morgan N. G. (2004). Expression and functional activity of PPARgamma in pancreatic beta cells. *British Journal of Pharmacology* **142**: 1162 - 1170.
8. Esmaeili, M. A. and Yazdanparast, R. (2004). Hypoglycaemic effect of *Teucrium polium*: Studies with rat pancreatic islets. *Journal of Ethnopharmacology* **95**: 27 - 30.
9. Cetin, Y. and Bullerman, L. B. (2005). Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay. *Food Chem. Toxicol.* **43**: 755 - 764.
10. Kimura, Y., Okuda, H. and Arichi, S. (1987). Effects of the extracts of *Ganoderma lucidum* on blood glucose level in rats. *Planta Medica* **54**: 290 - 294.
11. Hikino, H., Ishiyama, M., Suzuki, Y. and Konno, C. (1989). Mechanisms of hypoglycemic activity of Ganoderan B: A glycan of *Ganoderma lucidum* fruit bodies. *Planta Medica* **55**: 423 – 428.
12. Zhang, H. N., He J. H., Yuan, L. and Lin, Z. B. (2003). *In vitro* and *in vivo* protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage. *Life Sciences* **73**: 2307 – 2319.
13. Zhang, H. N. and Lin, Z. B. (2004). Hypoglycemic effect of *Ganoderma lucidum* polysaccharides. *Acta Pharmacology Sinica* **25**: 191 - 195.
14. Lin, J. Y., Chen, M. L., Chiang, B. L. and Lin, B. F. (2006). *Ganoderma tsugae* supplementation alleviates bronchoalveolar inflammation in an airway sensitization and challenge mouse model. *International Immunopharmacology* **6**: 241 - 51.
15. Zhang, J., Wang, G., Li, H., Zhuang, C., Mizuno, T., Ito, H., Mayuzumi, H., Okamoto, H. and Li, J. (1994). Antitumor active protein-containing glycans from the Chinese mushroom songshan lingzhi, *Ganoderma tsugae* mycelium. *Bioscience, Biotechnology and Biochemistry* **58**: 1202 - 1205.