ABSTRACT
This paper aims to disclose the hyperglycaemic activity of the polysaccharides extracted from *Morchella esculenta* (*M. esculenta*) mycelia. To this end, *M. esculenta* mycelia were cultured submerged fermentation, and the acidic polysaccharides were extracted and purified by anion exchange chromatography gel permeation chromatography. Then, a group of healthy rats was prepared, together with several groups of diabetes mellitus (DM) rats intervened with different doses of *M. esculenta* acidic polysaccharides. Next, several Morris water maze (MWM) tests were performed to reveal the regulation effect of these polysaccharides on the mRNA expression of the nerve growth factor (NGF) of the rats. The main results are as follows. The fasting blood glucose (FBG) and serum insulin (INS) of Group D (intervened by 600 μg·mL⁻¹ of polysaccharide) plunged to 8.12mmol/L and 31.67mU/L, respectively, compared to those of the control group (P<0.01), indicating that the polysaccharides can promote INS secretion. Meanwhile, the relative mRNA expression level of Group D grew by from the level of the control group (p<0.01). Thus, the acidic *M. esculenta* polysaccharide must have triggered the expression of NGF protein. The research findings lay a solid basis for the development of anti-hyperglycaemia food based on *M. esculenta* polysaccharides.

Key Words: Diabetes mellitus (DM), *Morchella esculenta* (*M. esculenta*), Acidic Polysaccharide, Nerve Growth Factor (NGF)

Introduction
Diabetes mellitus (DM) is a common endocrine disorder characterized by high blood sugar levels due to insulin deficiency. If left untreated, DM can cause many complications, including retina damage, cardiovascular disease, and degeneration of the peripheral and central nervous system (Han *et al*., 2018). As of 2015, an estimated 415 million people had diabetes worldwide, and the number is still increasing at a rapid rate.

Diabetic neuropathy (DNP) is the most common complication of the DM, which directly hinders the rehabilitation of DM patients. Nevertheless, there is no clear understanding of its cause or effective treatment to the disease. In recent years, the occurrence of the DNP has been attributed to the lack of neurotrophic factor, especially the nerve growth factor (NGF). The NGF is primarily involved in maintaining the growth and function of sympathetic and sensory nerves, regulating the physiological processes of target neurons and glial cells (Gang *et al*., 2003), and repairing traumatized tissues (Heng *et al*., 2003). The NGF has certain impacts on rapid physiological processes of hippocampus. For instance, Paredes *et al*. (2007) obtained data on the NGF role in neurotransmission within hippocampus, the relationship between the NGF...
and the brain-derived neurotrophic factor (BDNF), and basal glutamate and dopamine levels in the brain region. Some studies have revealed the close correlation between the lack of NGF and the occurrence and development of the DNP (Ge et al., 2002). Thus, the hope of curing the DNP lies in boosting the expression of NGF protein.

*Morchella esculenta* (M. esculenta) is an edible mushroom with high nutritional and medicinal values, e.g. the wealth of polysaccharides (Litchfield et al., 1963). Nowadays, *M. esculenta* is mainly cultivated through submerged fermentation, as it is very impractical to grow this rare and wild mushroom in traditional methods. Recent years has seen a boom in the artificial cultivation of the mushroom in China (Du et al., 2014). Some of the polysaccharides extracted from the fluid medium of *M. esculenta* are potentially bioactive (Wasser, 2002; Lei et al., 2016). These macromolecular polysaccharides can be broken down or bio-converted by intestinal bacteria, and improve the health of the host (Flint et al., 2012). Their metabolites enjoy remarkable physiological functions like cancer prevention, blood glucose reduction, and immunoregulation (Russell et al., 2013).

In light of the above, this paper extracts polysaccharides from *M. esculenta* mycelia and explores their regulation effect on the mRNA expression of the NGF of DM rats, with the aim to disclose the hyperglycaemic activity of these polysaccharides.

**Test results and analysis**

**Test materials and process**

The *M. esculenta* mycelia were cultured on a slant potato medium in a 500mL flask at 26°C for 5 days, and then processed by a rotary shaker at 100rpm. The lyophilized mycelia were placed in deionized water at 94°C for 0.5h to produce crude polysaccharides. Next, the total polysaccharides were precipitated by ethanol, deproteinized by Savage reagent, and washed with acetone and diethyl (Liu et al., 2016). The total polysaccharide was fractionated by DE52 anion exchange chromatography column (26mm×40mm) and Sephadex G-100 gel permeation chromatography column (2.0cm×60cm). The polysaccharide fractions were detected, collected, dialyzed and lyophilized.

Fifty male Sprague Dawley rats (200–250g each) were procured from the Laboratory Animal Centre, College of Animal Science, Jilin University, and kept at the temperature of 23±3°C, the relative humidity of 45% and a cycle 12h light/12h darkness throughout the experiment. Dry sawdust was changed every day to keep them clean.

Ten rats were randomly selected and allocated to the control group (n=10). Each of them was given basic feed for 8 weeks and injected with 0.50mL/kg sodium citrate-citric acid buffer. Each of the remaining 40 rats was given high-fat high-sugar diet for 8 weeks and injected with 15mg/kg streptozocin (STZ). After 2 days, the blood was collected daily from the angular vein of each rat.

The DM rat model was created with the level of fasting blood glucose (FBG) elevating between 11.1mmol/L and 33.3mmol/L. The DM rats were randomly divided into 4 groups: Group A (model control group), Group B (intervened by 200μg/kg *M. esculenta* polysaccharides), group C (intervened by 400μg/kg *M. esculenta* polysaccharides) and group D (intervened by 600μg/kg *M. esculenta* polysaccharides). The high-fat and high-sugar diet continued until the end of the experiment.

After feeding for 12 weeks, the rats were tested for learning and memory tasks with Morris water maze (MWM) for 5 consecutive days, aiming to evaluate their learning and memory capacities (Liu et al., 2013).

Prior to the MWM test, the rats were fasted overnight; the blood samples were taken from the sublingual vein, centrifuged at 3,000rpm for 10min, and kept at -80°C after 10min standing to clot. Then, the FBG and serum insulin (INS) levels were measured. Meanwhile, the rat brain was carefully extracted, rinsed with ice-cold 0.9% sodium chloride solution, and homogenized in 50mmol/L phosphate buffer at the pH of 7.4 (1:5 w/v). The homogenate was then centrifuged at 3,000 rpm for 20min at 4°C to separate cell debris for biochemical determinations (Motawi et al., 2017).

The total RNA was isolated from rat’s brain tissue by TRIzol reagent (Sigma) following the instructions. The complementary DNA was synthesized and reverse transcribed by the RT-qPCR kit (Sigma). The RNA concentration and purity were measured by optical absorbance (Abs) at 260nm and 280nm.
The mRNA expression levels for β-actin and NGF were captured through RT-PCR. For quantitative analysis of mRNA of β-actin and NGF, the primers adopted for the reaction and β-actin gene taken as endogenous reference were designed by Primer 5.0.

Each experiment was repeated three times, and the results were expressed as means ± standard deviation for triple determinations. Variance analysis and t-tests were performed to identify the difference in means by one-way analysis of variance (one-way ANOVA) on IBM SPSS software 18.0. The significance level was set at P<0.05 and the highly significance level was set at P<0.01.

**Extraction and purification of polysaccharides**

The crude polysaccharides extracted from *M. esculenta* were fractionated on DE52 anion exchange chromatography column to produce acidic polysaccharide. Sodium chloride solution, and the minor peak was eluted with deionized water. According to the yield, the major fraction containing acidic polysaccharide was collected for further purification on Sephadex G-100 gel permeation chromatography column (Figure 2). Then, acidic polysaccharide was collected from the first single elution peak, and turned into purified form through concentration, dialysis and lyophilization (yield: 66.3%). The purified acidic polysaccharide was reserved for subsequent analysis. For the acidic polysaccharide collected from the second peak, the yield (9.4%) is too low to support further analysis on physiological activity.

**Molecular weight**

Suresh et al., (2013) pointed out that the molecular weight (Mw) of polysaccharides directly bears on their physiological activities, especially pharmacological activities. The polysaccharides with low Mw boasts stronger penetrability through biological tissues, and thus better curative effect, than high-molecular weight polysaccharides, while the polysaccharides with high molecular weight often has no physiological activity (Jian et al., 2001).

After purification on Sephadex G-100 (Figure 3a), a single narrow symmetrical peak (Figure 3b) appeared on the chromatogram of *M. esculenta* acidic polysaccharide. The Mw was determined by the GPC-RI system and calculated on work station. The results show that the molecular weight obeys a rather narrow distribution, which confirms the homogeneity of the polysaccharide sample.
The two components (Figure 3a) thus obtained are denoted as PI and PII, respectively, and their weight-average molecular weights were 222,344Da and 428Da (Table 1), respectively. The molecular weight of PI was so small that the component was determined as oligosaccharide or monosaccharide. There might be a small amount of oligosaccharide impurities in the sample. Thus, only the PI fraction was collected for purity and yield calculation.

The single narrow symmetric peak of PI was prepared by GPC (Figure 3b). It can be seen that PI is homogenous in terms of $M_w$. Then, the purified PI was collected, dried, and coloured in light yellow. As calculated by work station, the polydispersity index (PDI) of PI stood at 1.11758, indicating that the molecular weight is distributed in a rather narrow range. The actual preparation yield was 45.29% and the polysaccharide content was 92.12%.

### Table 1. Yields and molecular parameters of M. esculenta acidic polysaccharide in GPC analysis

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Retention time/min</th>
<th>Yield/%</th>
<th>$M_n$/Da</th>
<th>$M_w$/Da</th>
<th>$M_w/M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>13.656</td>
<td>77.694</td>
<td>198,950</td>
<td>223,344</td>
<td>1.11758</td>
</tr>
<tr>
<td>PII</td>
<td>20.218</td>
<td>22.306</td>
<td>413</td>
<td>428</td>
<td>1.03685</td>
</tr>
</tbody>
</table>

$M_n$: number-average molecular weight; $M_w$: weight-average molecular weight; $M_w/M_n$: polydispersity

### MWM tests

After 5 days of continuous training on the learning and memory tasks, the rats of the control group could find the location of the platform quickly. By contrast, Group A rats spent a long time and swam a long distance before finding the platform. This means the STZ-induced DM had successfully weakened the memory of the rats. The rats in Groups B–D were much better at memory and learning than those in Group A, especially Group D.

### Table 2. MRM transitions of DM rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Escape latency/s</th>
<th>Crossing number/120s</th>
<th>Swimming distance/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.72±2.57a</td>
<td>2.23±0.19a</td>
<td>2247.25±17.25a</td>
</tr>
<tr>
<td>A</td>
<td>59.77±8.26**</td>
<td>1.45±0.56**</td>
<td>456.30±20.98**</td>
</tr>
<tr>
<td>B</td>
<td>51.18±6.61**</td>
<td>2.09±0.33**</td>
<td>860.62±25.59**</td>
</tr>
<tr>
<td>C</td>
<td>40.27±4.24**a</td>
<td>2.70±0.61**a</td>
<td>1620.00±32.72**a</td>
</tr>
<tr>
<td>D</td>
<td>32.35±3.02**a</td>
<td>2.67±0.52**a</td>
<td>2096.25±24.00**a</td>
</tr>
</tbody>
</table>

*: P<0.05 vs Control group; **: P<0.01 vs Control group; *: P<0.05 vs A group; **: P<0.01 vs A group

Escape latency was observed among rats in each group (Table 2). In the hidden platform test, the escape latency of Group A rats was as high as 59.77s, highly significantly longer than that of the control group rats (30.72s) (p<0.01). Compared with Group A rats, the rats in Groups B–D exhibited much lower escape latencies, a signal of the escape latency reduction effect of *M. esculenta* acidic polysaccharide. Moreover, the reduction effect gradually increased with the dose of polysaccharide. The rats fed with medium dose (Group C) and high dose (Group D) had much lower escape latency than that of Group A rats (p<0.01), and similar escape latency as that of control group rats (p>0.05). Compared with Group A, the 600 g/kg polysaccharide in Group D reduced the escape latency from 59.77s to 32.35s. Hence, high dose of polysaccharide can effectively inhibit STZ-induced memory damage, and protect the brain tissue from injuries.

### FBG and INS levels

Since the establishment of the DM rat model in week 8, the FBG was measured weekly by Roche glucose meter until the experiments ended in week 12 (Chen et al., 2009) (Table 3). In week 9, the FBG of Group A was significantly higher than that of the control group. On the contrary, the FBGs of DM rats in Groups B–D were all lower than the level of the control group, thanks to the intervention of *M. esculenta* acidic polysaccharide. In week 12, the FBG in Group D (high dose group) plunged to 8.12mmol/L compared with Group A (P<0.01). These results demonstrate that the polysaccharide has a therapeutic effect on hyperglycaemia of DM rats, and can significantly reduce the FBG in rats. Meanwhile, the INS level in Group D (high dose group) recovered to 31.67mU/L, contrary to that of Group A (P<0.01). This means the polysaccharide can promote INS secretion and elevate the level.

### mRNA expression of NGF

The effect of *M. esculenta* acid polysaccharide on mRNA expression level of NGF was analysed by RT-PCR. As shown in Figure 4, with the intervention of 600 μg·mL$^{-1}$ of polysaccharide, the relative mRNA expression level of NGF in Group
D grew by 3.25-fold from the level of Group A (p<0.01). Thus, *M. esculenta* polysaccharide must have triggered the expression of NGF protein and that the expression of β-actin protein was homogeneous.

**Figure 4.** Effect of different contents of *M. esculenta* acidic polysaccharide on mRNA expression level of NGF

* P < 0.05 vs A group; ** P < 0.01 vs A group

### Conclusions

In our research, the DM model rats were created through high-fat diet and the STZ (Chen et al., 2014). The established model was proved valid because the FBG levels in DM groups were significantly higher than those in the control group. Through comparison and discussion, it is concluded that *M. esculenta* acidic polysaccharide with molecular weight of 222,344Da could restore the blood insulin content in hyperglycaemic rats, boost the expression of NFG, and enhance the cognitive ability of model rats. Suffice it to say that the intervention of *M. esculenta* acidic polysaccharide can effectively restore serum adiponectin level, FBG value, and insulin secretion in DM rats. The research findings lay a solid basis for the development of anti-hyperglycaemia food based on *M. esculenta* polysaccharides.

### Acknowledgments

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