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RESEARCH ARTICLE **OPEN ACCESS**

ANTI-DIABETIC AND ANTIOXIDANT ACTIVITY OF *BETULA HIPPOLYTII* AND *BETULA PLATYPHILLA*

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ABSTRACT

Birch leaves were used as a substitute for birch bark, buds, and Chaga of birch. In traditional medicine, birch leaves are considered to have less toxic. Numerous researches conducted in Russia, Bulgaria, Japan, and China on *Betula pubescens* Ehrh., *Betula pendula* Roth., *Betula rezniczenkoana* (Litv.) Schischk., *Betula humilis* Schrank., *Betula mandshurica* (Regel) Nakai found that birch barks and leaves contain antioxidants, and they have anti-cancer, anti-yeast, antibacterial and anti-inflammatory properties, protect the liver and promote bile secretion. The studies showed that the medicinal plants had anti-inflammatory on stomach inflammation during extreme stress and promoted bile secretion. The birch leaf Phyto preparations used in experimental animals reduced insulin resistance of peripheral tissues and lowered blood glucose. It was noted in Traditional Mongolian Medicine that the birch bark was used to treat inflammation for acute diseases. The aim of this study was to determine the effect of two species of birch leaves on blood glucose and antioxidant activities in diabetic rats. The study was conducted at the Drug Research Institute of the Monos Group. 40 WISTAR non-linear white rats weighing 150-204 g, dry extract of birch leaves of the two species (Alloxan monohydrate Tokyo Chemical Industry LTD), IGM-100 3A blood glucose meter (Blood glucose test meter, Infopia LTD, Brussels Belgium), glucose test (Blood glucose test strip only), antioxidant activity kit (Rat Malondialchehyche Elisa KIT, cat. № EKRAT- 0266, Jilin province, China). The blood glucose level of the pathological control group rats reduced from 31.5 mmol/l to 17.1 mmol/l in 14 days. The blood glucose levels of *Betula platyphylla* group lowered to 6.3 mmol/l; *Betula hippolytii* group's lowered to 6.9 mmol /l. The results showed that the maximum MDA level was determined from the control group, *Betula hippolytii* and *Betula platyphilla* groups. The maximum MDA rate was reduced by 33.9% and 53.5%, respectively for *Betula hippolytii* and *Betula platyphilla* groups. *Betula hippolytii* birch leaves and *Betula platyphilla* birch leaves have been shown to lower blood glucose levels and have antioxidant activity for diabetic animals.

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INTRODUCTION

The birch trees are cold resistant and mostly grow in mountainous areas where 600-1200 meters above sea level (Ulziikhutag, 1985; Volodya *et al*., 2010). Many different species of birch grow (Khurelbaatar *et al*., 2018) in the northern Europe (Molocovskhii *et al*., 2006), Asia and North and South America and in countries such as Russia (Enkhjargal *et al*., 2018; Safanov, 2005), Bulgaria (Penkov *et al*., 2015), Kazakhstan, Japan (Hiroyuki *et al*., 1996), South Korea, and Mongolia (Ligaa, 2006). 12 species of birch grow in Mongolia and they constitute about 20% of the country's green forest (Grubov, 2008). It was determined that the birch barks and leaves have high antioxidant, anti-cancer, anti-yeast infection, anti-bacterial and anti-inflammatory properties, protect liver and promote bile secretion (Ligaa, 1997, 2006). According to the research (Abyshev *et al*., 2007; Penkov *et al*.,2015) conducted on the birch leaves, the leaves had anti-inflammatory effect on the test animals with carrageenan (Molocovskhii *et al*., 2006) and formaldehyde induced inflammation, protected stomach linings during extreme stress, promoted bile secretion and had antigiardiasis

effects. Furthermore, the phytomedicine made from birch leaves solution was used in adrenalin induced hyperglycemia and alloxan induced diabetes, it was discovered that the medicine reduced the insulin tolerance of peripheral tissue (Enkhjargal *et al*., 2018; Ossipov *et al*., 1996) and blood glucose level (Ligaa, 1999; Penkov *et al*., 2015). *Betula pubescens*, *Betula pendula*, *Betula rezniczenkoana*, *Betula humilis*, *Betula mandshurica* birch species grow in Russia, Bulgaria, Japan, and China and their barks found to have anti-oxidant, anti-cancer, anti-yeast, and anti-bacterial effects and protected liver and promoted bile secretion. The birch barks' inner and outer layers contain biologically active compounds, and birch leaves and young branch's water and alcohol solutions are used in traditional medicines to treat urine tract problems and rheumatism (Ligaa, 1999, 2006). In the recent years, many medicines have been extracted from birch branches that are used to treat liver and gallbladder problem, vitamin deficiency, atherosclerosis, stomach inflammation and antiseptics (Volodya, 2014; Academy of Sciences of the USSR, 1986).

In traditional Mongolian medicine, *B. rezniczenkoana* bark was used to reduce fever, relieve toxicity, support urination and increase blood flow; the birch leaves' water extract was used to treat liver and hot gallbladder disease (Volodya, 2014; Ligaa 1999, 2006). It was noted that *B. hippolytii*, *B. platyphylla*, *B. mandshurica*, *B. microphylla*, *B. humilis* birches had medicinal properties and their barks, buds (Ligaa, 2006), sometimes *Inonotus obliguus* (chaga mushroom) was used instead of birch leaves in medicines. According to a study conducted in 2005, 8.2% of Mongolia's population had diabetes. In the last 20 years, the number of diabetic patients increased due to the slowly changing lifestyle, asymptomatic diabetes, and the late diagnosis. Therefore, it is important to study the birch leaves which have been used in Mongolian traditional medicine to treat liver diseases and study its pharmacology effect. The aim of this study was to determine *B. platyphylla* and *B. hippolytii* leaves effects on blood glucose level and antioxidant activity of diabetic rats.

MATERIALS AND METHODS

Research tools: 40 WISTAR, non-linear white rats weighing 150-204 g were used in the experiments. Dry extract of birch leaves of the two species (Alloxan monohydrate Tokyo Chemical Industry LTD), IGM-100 3A blood glucose meter (Blood glucose test meter, Infopia LTD, Brussels Belgium) and glucose test (Blood glucose test strip only, province, China) were used for the experiment. Lenzen's (2008) method (Lenzen, 2008; Lukenes, 1948) was used to induce Alloxan in the rats and the antioxidant properties were determined by the antioxidant activity kit (Rat Malondialchehyche Elisa KIT, cat. № EKRAT- 0266, Jilin)

Research methods: The birch leaves extracts were extracted by 1:10 proportionate 70% acetone, later, the thick-extract was collected from 50-60^{0}C vacuum vaporizer and dried by a diffuser.

Diabetes induction method in the rats: 175 mg/kg alloxan was delivered in the lower pelvic hypodermis of 40 WISTAR, non-linear white rats weighing 150-204 g.

Determining antioxidant activity: Dried extracts of leaves antioxidant level were determined using *in vitro* method. 0.2 ml *v.orbitalis* blood serum was used in MDA kit (Rat Malondialchehyche Elisa KIT, cat. № EKRAT- 0266, Jilin). Pharmacology experiment followed "Ethical Guidelines for the Use of Animals in Research". The statistical analysis was conducted using SPSS 20 software and the significance of difference between the groups was evaluated by the Student's T statistics and p-value below 0.05 was considered statistically significant.

RESULTS

The result of the study to determine blood glucose level using *in vivo* method: 175 mg/kg alloxan was determined by body ratio (35 ml/200 g) and it was used to induce diabetes in the rats. The rats were divided into 4 groups and each group contained 10 rats: 2 control group (healthy and sick) and 2 treatment group (version1 and version 2). Treatment group 1 was orally given 59 mg/200g of *B. hippolytii* and *B. platyphylla* leaves dry extracts by stylet for 14 days. The result is shown in Figure 1.

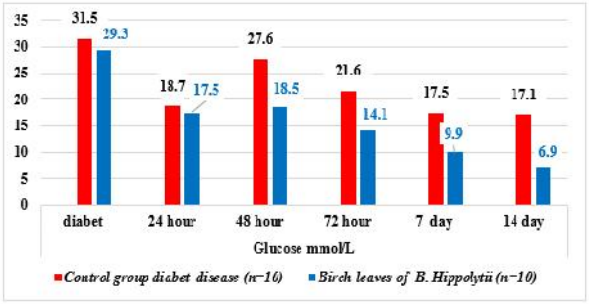

Figure 1. The blood glucose (mmol/l) reducing effect of the birch hippolytii (*B. hippolytii*) leaves

Treatment group 1: *B. hippolytii* leaves extract helped to reduce blood glucose level after 14 days of use. The blood glucose level of treatment group 1 that used *B. hippolytii* extract reduced by 1.2 mmol/l (6.4%) in the first 24 hours, 9.1 mmol/l (32.9%) in 48 hours, 7.5 mmol/l (34.7%) in 72 hours, 7.6 mmol/l (43.4%) in 7 days and 10.2 mmol/l (59.6%) in 14 days compared to the pathological control group.

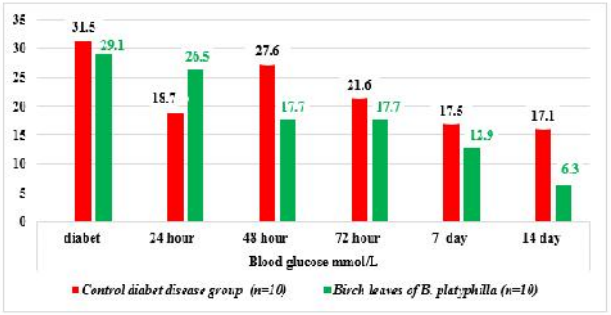

Figure 2. The blood glucose (mmol/l) reducing effect of the flat leaved (*B. platyphilla*) birch leaves

Treatment group 2: The blood glucose level of treatment group 1 that used *B. hippolytii* extract reduced by 7.8 mmol/l (29.4%) in the first 24 hours, 9.9 mmol/l (35.9%) in 48 hours, 3.9 mmol/l (18.1%) in 72 hours, 4.6 mmol/l (26.2%) in 7 days and 10.8 mmol/l (63.2%) in 14 days compared to the pathological control group.

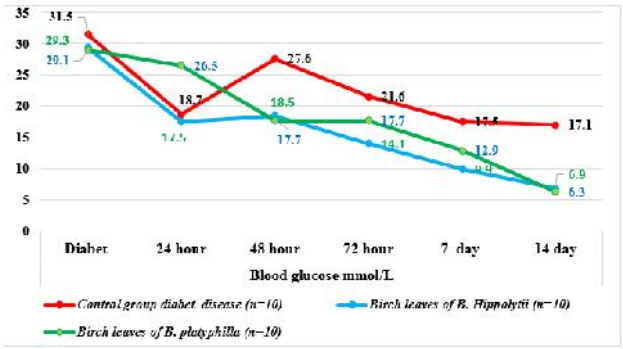

Figure 3. Result comparing of blood glucose reducing effect the by standard error in two treatment groups birch leaves (p<0.05)

Based on the study results, the blood glucose levels of rats that were given *B. hippolytii* leaves extract were stable during the first 24-48 hours and from 48 hours to 14 days of the treatment, it was reduced to 6.9 mmol/l *(p<0.05)*. On the contrary, *B. platyphylla* did not reduce the blood glucose level in the first 24 hours, and from 24 to 48 hours of treatment, it rapidly reduced the blood glucose and stabilized between 48 to 72 hours and from 72 hours to 14 days treatment, the blood glucose level was reduced to 6.3 mmol/l *(p<0.05)*. During the study, the blood glucose level of the controls groups were between 31.5 mmol/l and 17.1 mmol/l, and as for treatment groups, *B. platyphylla* group's blood glucose level was 6.3 mmol/l and *B. hippolytii* was 6.9 mmol/l. The study results showed that the birch leaves' dry extracts had anti-diabetic effect.

The result of antioxidant activities: After 14 days of 59 mg/200 g birch leaves' extract treatment, the antioxidant levels were determined using *in vitro* method. 1 ml blood was drawn from *v.orbitalis* and 0.2 ml serum was extracted and analyzed by MDA kit (Rat Malondialchehyche Elisa KIT, cat. № EKRAT- 0266, Jilin). MDA level in healthy rat′s blood serum was 0.3 nmol/ml at the lowest and 4.8 nmol/ml at the highest. The level was 3.76 nmol/l in the pathological control group rats which showed that antioxidant process was disturbed by alloxan.

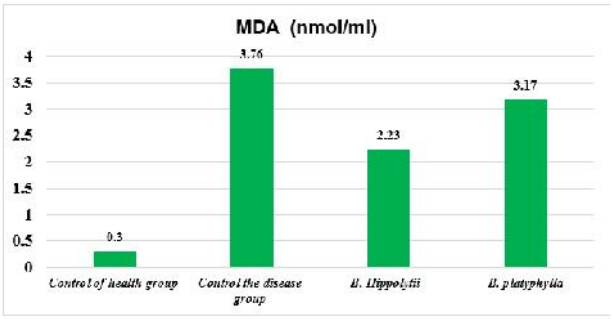

Figure 4. Antioxidant activity of the extract birch leaves

The treatment group's MDA level's maximum amount was 1.04 nmol/ml (21.7%) which was lower than the maximum level of control groups and the anti-oxidant level regained after 14 days treatment Treatment group 1: the group that used *B. hippolytii's* MDA level was 2.23 nmol/ml and compared to that of control group, it was reduced by 1.53 nmol/ml (41.7%). Compared to the maximum MDA level of the control group, the group that used *B. hippolytii* leaves extract was reduced by 2.23 nmol/ml (53.5%). The group that used *B. platyphylla*'s MDA level was 3.17 nmol/ml and compared to that of pathological control group, it was reduced by 0.59 nmol/ml (15.7%). Compared to the maximum MDA level of control group, the group that used *B. platyphylla* leaves extract was reduced by 1.63 nmol/ml (33.9%). The study results showed that the MDA level of *B. hippolytii* and *B. platyphylla* leaves groups were reduced by 33.9% and 53.5% respectively. This shows that the birch leaves had antioxidant effects.

DISCUSSION

Using 59.0 mg/200g of *Betula hippolytii* and *Betula platyphylla* leaves dry extracts for 14 days as a treatment have shown to have anti-diabetic effect and it can be explained that the activation of protein kinase B ferment stabilized GLUT4 transporter (Enebish, 2012). Alloxan harmed selected β-cells of gallbladder to induce diabetes and β-cells lose their antioxidant properties. Alloxan GLUT2 moves and accumulates in β-cell via glucose transporter protein (Lenzen, 2008; Lukenes, 1948). The some substances in the cell for example, the substances with thiol group forms a liberated compound, such as from glutathione oxidation-reduction reaction is involving dialuric acid. The dial uric acid undergoes oxidized reaction so produced oxygen active forms such as superoxide and hydrogen peroxide (Batgerel, 2012). These active forms are attending by the irons ion and producing hydroxyl radicals (Odontuya, 2018). The hydroxyl radicals reduce the amount of antioxidant compounds (Selenge and Odontuya, 2019).

MDA level in healthy rat's blood serum was 0.3 nmol/ml at the lowest. The antioxidant effect is measured by how much the MDA is reduced compared to the maximum amount. The MDA level was 3.76 nmol/l in control group sick rats which showed that antioxidant process was disturbed by alloxan. Comparing the MDA level of control group sick rats to *B. hippolytii* and *B. platyphylla* treatment group, the MDA levels dropped by 1.53 nmol/ml (40.7%) and 0.59 nmol/ml (15.7%) respectively. This shows that the birch leaves have antioxidant effects. The anticarcinogenic, anti-mutagenic and cardio protective effects reported are generally associated with the flavonoids' antioxidant properties. Flavonoids as reducing agent and donors of hydrogen and free radical scavengers (Aaby *et al*., 2004).

Conclusion

The following conclusions were made based the study results. According to *in vivo* study results, the blood glucose level of the *B. platyphylla* group and the *B. hippolytii* group reduced to 6.9 mmol/l (14%) and 6.3 mmol/l (22%) respectively. This showed the birch leaves dry extracts had anti-diabetic effects. According to *in vitro* study results, the MDA in blood serum decreased by 2.23 nmol/ml (53.5%) in the *B. hippolytii* group and 3.17 nmol/ml (33.9%) in the *B. platyphilla* group. This showed that the birch leaves had antioxidant effects.

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