

EFFECTS OF WHITE MULBERRY (*MORUS ALBA*) LEAF TEA INVESTIGATED IN A TYPE 2 DIABETES MODEL OF RATS

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Abstract: The present study was aimed to investigate the anti-diabetic effects of a low (0.25%) and a high (0.5%) dose of white mulberry leaf tea in a rat model of type 2 diabetes (T2D). Six week old male Sprague-Dawley rats were divided into four groups: Normal control (NC), Diabetic control (DBC), Diabetic mulberry tea low (DMTL, 0.25%) and Diabetic mulberry tea high (DMTH, 0.50%). T2D was induced by feeding a 10% fructose solution in drinking water for 2 weeks to induce insulin resistance, followed by a single injection (*i.p.*) of streptozotocin (40 mg/kg body weight (b.w.)) to induce partial pancreatic β -cell dysfunction in all groups except the NC group, which only received a normal drinking water and citrate buffer (pH 4.4) instead of fructose solution and STZ injection, respectively. After 4 weeks feeding of brewed mulberry leaf tea, there were no significant improvements in polyphagia, polydipsia, body weight gain, blood glucose, glucose intolerance, serum insulin, fructosamine, AST, ALT, creatinine, albumin and uric acid levels and liver parameters when serum total cholesterol was significantly and LDL-cholesterol and triglyceride concentrations were markedly decreased in the DMTH group compared to the DBC and DMTL groups. Serum total proteins were significantly reduced in DMTL and DMTH groups compared to the DBC group. These results suggest that brewed white mulberry leaf tea has hypolipidemic rather than antidiabetic effects at least in this experimental condition. However, the effects of the different brands of white mulberry leaf tea may be varied due to various factors.

Keywords: mulberry leaf tea, *Morus alba*, type 2 diabetes, rats

Diabetes is an ongoing public health concern that has an increasing prevalence globally. According to International Diabetes Federation (IDF), at least 371 million people have diabetes worldwide in 2012, which is predicted to be 552 million by 2030 (1). Of all the diabetic cases, 90–95% of them suffer from type 2 diabetes (T2D) (2). Unlike type 1 diabetes (T1D), which is denoted as diminished insulin production, T2D is a heterogeneous disorder characterized by impaired cellular responses to insulin known as insulin resistance and followed by progressive partial pancreatic β -cell dysfunction (3). Due to insulin resistance, pancreatic β -cells secrete abnormally high levels of insulin in order to control blood glucose levels, however overtime, hyperglycemia, hyperinsulinemia, pancreatic β -cell dysfunction and subsequent progressive pancreatic β -cell destruction occur (4–6). Nowadays, diet and lifestyle choices tend toward high caloric intake and low physical activity, pro-

moting the development of obesity. An increased risk of developing T2D has been linked to obesity through its association with diets of high caloric content (7). Since T2D is characterized by 2 main pathogenesis; insulin resistance and partial pancreatic β -cell dysfunction (4), these are also the two predominant targets for disease control.

Despite the wide availability and range of existing anti-diabetic drug therapies, there is still an ever-growing need for better and/or alternative therapies to combat the rising numbers of global diabetic patients. Furthermore, the demand for new alternative therapies is increasing as patients search for cheaper options, wider availability as well as to avoid the dissatisfactory symptoms and consequences of traditional drug therapies, including weight gain, hypoglycemia or certain contraindications that may limit their use (8, 9).

White mulberry (*Morus alba*) has been used over the centuries in traditional Chinese medicine as

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a common agent to treat a variety of conditions including diabetes, atherosclerosis, cancer as well as for boosting the immune system through potent antioxidant activity (10). Different parts of the mulberry plant (fruit, bark, leaf and root) have drawn interest in their role to treat diabetes, when the root and bark often was used to reduce hyperglycemia (11). Several studies have already investigated various alkaloids, flavonoids and phytochemicals in white mulberry, having found especially in leaves to exhibit anti-diabetic effects. These effects include inhibition of α -glycosidase, sucrase and maltase enzymes activity (12, 13), reducing carbohydrates metabolism and thus lowering blood glucose levels (12), prevention of lipid peroxidation (14), improvement of dyslipidemia, especially hypercholesterolemia (15) and inhibiting oxidation of LDL cholesterol (16). Kimura et al. (17) found 1-deoxynojirimycin (DNJ), an alkaloid in white mulberry leaf and reported to have non-fasting blood glucose (NFBG) lowering activity in humans. Nowadays, white mulberry leaf extracts are found in various food products especially sold as a tea and is readily available in many countries (15). Whilst all current research investigates white mulberry leaf extracts, none investigate the effect of white mulberry leaf tea, as consumed by humans, either in humans or experimental animals.

Hence, the present study was conducted to investigate the anti-diabetic effects of a low (0.25%) and a high (0.5%) dose of brewed white mulberry leaf tea in a T2D model of rats.

MATERIALS AND METHODS

Reagents and materials

Streptozotocin (STZ) (> 98%) (Sigma-Aldrich) was purchased from Capital Lab Supplies cc. Durban, South Africa. Fructose (Nature's Choice™ Wholefood specialists, Meyerton, South Africa 1960) was purchased from a local pharmacy. White mulberry leaf tea was purchased from Beautique Thai (Thailand). A glucometer (GlucoPlus Inc., Quebec, Canada) with a maximum measuring capacity of 600 mg/dL) was used for measuring blood glucose levels.

Animals

Twenty eight (6 weeks old) male Sprague-Dawley rats (mean body weight 191.88 ± 16.40 g) were procured from the Biomedical Resource Unit (BRU) at Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. Animals were randomly subdivided into 4 groups of 6–7 rats in each group as follows: Normal control (NC),

Diabetic control (DBC), Diabetic culberry tea low (DMTL, 0.25%) and Diabetic mulberry tea high (DMTH, 0.50%). Two rats per polycarbonated cage were housed in a temperature and humidity controlled room with a set of 12 h light-dark cycle. The rats were fed a commercially available rat chow diet *ad libitum* throughout the 7 week experimental period. The animals were maintained according to the rules and regulations of the University of KwaZulu-Natal (UKZN) Animal Ethics Committee (Ethical approval number: 029/11/Animal).

Induction of diabetes

T2D was induced in the animals in DBC, DMTL and DMTH groups by feeding 10% fructose solution for the first 2 weeks followed by an injection (*i.p.*) of STZ (40 mg/kg b.w.) dissolved in citrate buffer (pH 4.4) when the animals in NC group were fed with normal drinking water and injected with citrate buffer, respectively (18). Non-fasting blood glucose (NFBG) levels of all animals were measured 1 week after STZ injection by using a portable glucometer in the blood collected from tail veins. Animals with a NFBG level > 300 mg/dL were considered as diabetic.

Tea preparation and intervention

White mulberry leaf tea was prepared in the following concentrations: 0.25 and 0.5%. According to the concentration, tea bags were brewed exactly for 10 min in boiling water, cooled to room temperature and supplied to the respective group of animals *ad libitum* during 4 weeks intervention period, starting from one week after the STZ injection. At the same time, the animals in the NC and DBC groups were supplied with normal drinking water instead of mulberry tea. Daily food and fluid intake and weekly body weight changes and NFBG were measured during the entire intervention period.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test (OGTT) was performed in all animals in the last week of the 4 week intervention period. In order to perform this test, after an overnight fast (12 h), rats were orally dosed with a D-glucose solution (2.0 g/kg b.w.) and glucose concentrations were subsequently measured in the blood collected from the tail veins at 0 (just prior to oral glucose dosing), 30, 60, 90 and 120 min after oral dosing of glucose.

Collection of blood and liver

At the end of the experimental period, animals were fasted for 14 h and sacrificed using halothane

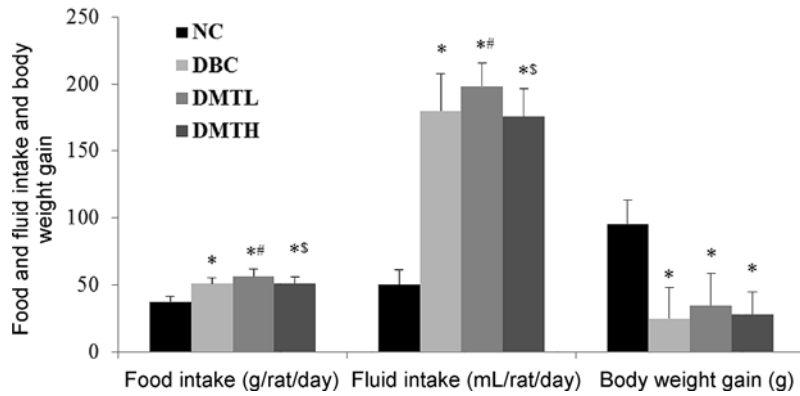


Figure 1. Food and fluid intake and body weight gain in different animal groups at the end of the experimental period. * $p < 0.05$ vs. NC, # $p < 0.05$ vs. DBC, \$ $p < 0.05$ vs. MTL (Tukey-Kramer's multiple range *post-hoc* test)

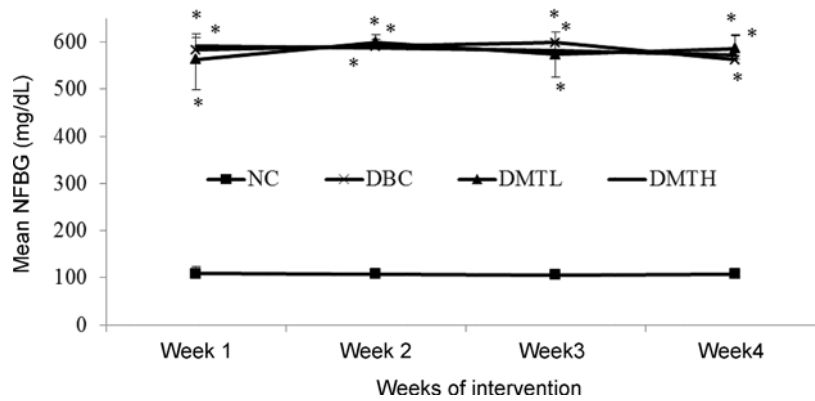


Figure 2. Non-fasting blood glucose (mg/dL) of different rat groups during the intervention trial period. * $p < 0.05$ vs. NC (Tukey-Kramer's multiple range *post-hoc* test).

euthanasia after which blood and liver were collected. Blood was collected through cardiac puncture and immediately placed into heparinized tubes and preserved on ice. The blood samples were centrifuged at 3000 rpm for 15 min and separated serum was stored at -30°C for further analysis. Liver samples were washed in cold saline, wiped dry with filter paper, weighed on an analytical balance and preserved at -30°C for subsequent analysis.

Analytical methods

Liver glycogen concentration was measured photometrically by using phenol-sulfuric acid method as described by Lo et al. (19). Serum insulin concentration was measured using an ultra-sensitive

rat insulin ELISA kit (Mercodia AB, Uppsala, Sweden) in a multi-plate ELISA reader (Biorad-680, BIORAD Ltd., Japan). Serum lipid profiles, serum creatinine, total proteins, serum albumin, fructosamine and liver function enzymes (AST and ALT) were measured using an Automated Chemistry Analyzer (LabmaxPlenno, Labtest, Costa Brava, Lagoa Santa, Brazil).

Statistical analysis

All data are presented as the mean $\pm$ SD. The data were analyzed by a statistical software package (SPSS version 18) using the Tukey's HSD multiple range *post-hoc* test. The values were considered significantly different at $p < 0.05$.

RESULTS

During the experimental period, food intake and fluid intake in DMTL, but not DMTH group was significantly higher than the DBC group. Mean body weight gain amongst the groups was not significantly different from each other, except from that of the NC group (Fig. 1).

The DMTL and DMTH groups showed no significant improvement either for NFBG over 4 weeks (Fig. 2) when glucose tolerance ability was worsened by the feeding mulberry leaf tea (Fig. 3).

There was no significant difference in liver weight amongst the groups (Table 1); however, relative liver weights and liver glycogen concentrations were significantly higher in the diabetic groups

compared to the NC group. The liver function enzyme ALT was significantly higher in both DBC and DMTH compared to the NC group, whereas not in the DMTL group (Table 2). No significant difference was observed for serum AST and creatinine concentrations among the groups, however, serum total proteins were significantly lower in DMTL and DMTH compared to DBC, as well as serum albumin was significantly reduced in DMTL and DMTH compared to NC, but not DBC group. Serum uric acid was significantly lower in the DMTL group compared to the NC group when no difference observed among the other groups (Table 2). Serum insulin and fructosamine concentrations were also not affected by the consumption of the either dosages of mulberry leaf tea (Table 2).

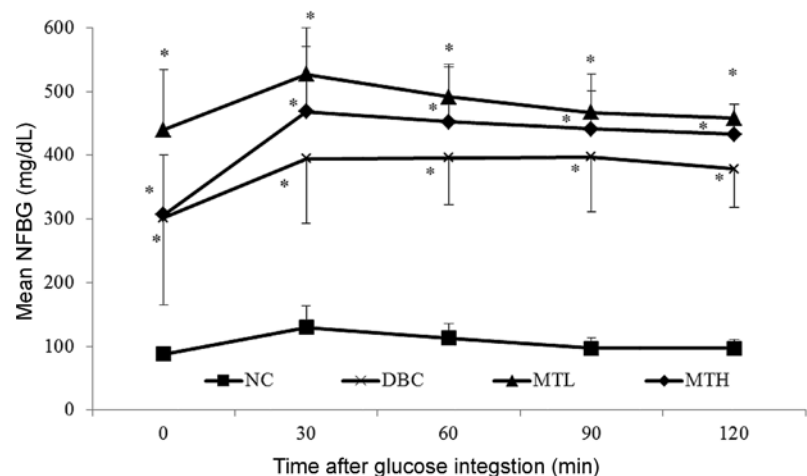


Figure 3. Oral glucose tolerance test over a 2 h period.*p < 0.05 vs. NC (Tukey-Kramer multiple range *post-hoc* test)

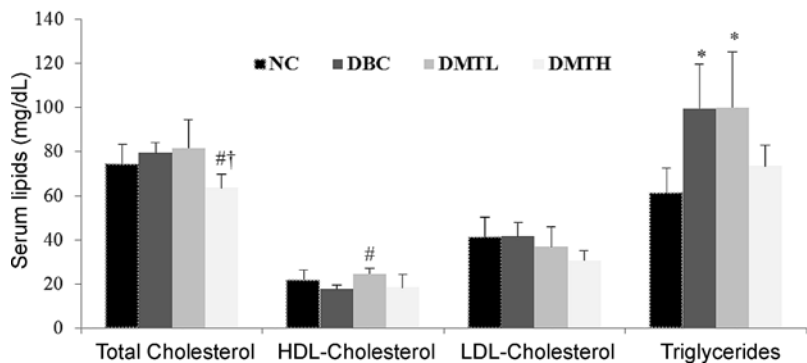


Figure 4. Serum lipid profile of the different animal groups at the end of the experimental period *p < 0.05 vs. NC, #p < 0.05 vs. DBC, †p < 0.05 vs. DMTL (Tukey-Kramer's multiple range *post-hoc* test)

Total cholesterol was significantly lower in the DMTH group compared to DBC and DMTL groups, whereas HDL was significantly higher in DMTL compared to DBC group. Serum triglycerides were significantly higher in DBC and DMTL compared to the NC group, however, comparatively lower serum triglyceride and LDL-cholesterol concentrations were observed in the DMTH group compared to the DBC and DMTL groups (Fig. 4).

DISCUSSION

Due to the rapidly rising numbers of T2D patients, intensive research on diabetes therapy and prevention has similarly increased. Currently, oral drug therapies for T2D focus on improving insulin secretion and improving insulin sensitivity (20). Ideal alternative therapies must exhibit a similar degree of efficacy compared to conventional drug therapy, however without the negative side effects often associated with the conventional therapies (21). In late 1980's, with over 400 reported traditional medicines for the treatment of T2D, the World Health Organization Expert Committee on Diabetes has recognized the great potential of natural plants and functional foods as alternative treatments for

T2D and recommended to carry our further investigations on them (22). Today, the plant based individual alternative or isolated therapy of T2D is very common and popular in most developing and even in some developed countries.

Tea in traditional Asian medicine is believed to promote both good health and longevity (23). In recent years, leaves from the mulberry plant have gained much popularity as a tea drink for diabetics in many Asian countries (10). The major anti-diabetic compound in white mulberry leaves is a glucose analogue called 1-deoxynojirimycin (DNJ), which inhibits the intestinal enzyme α -glucosidase by binding to the active site in the enzyme (24). α -Glucosidase is considered to be one of the most important enzymes in starch digestion in the small intestine (25) and thus DNJ is believed to be responsible for the reduction in NFBG and hyperglycemia by reducing the rate of both carbohydrate and lipid absorption (24). It has been reported that a minimal dose of mulberry DNJ (6 mg/60 kg b.w.) is required to reduce NFBG and decrease insulin secretions in human subjects (17, 26).

Recently, Vichasilp et al. (26) investigated 35 mulberry tea varieties in Thailand to determine and develop a mulberry tea blend with the optimal DNJ

Table 1. Liver weight, relative liver weight, liver glycogen and liver function enzymes of the different animal groups at the end of the experimental period.

Rat groups/ liver parameters	NC	DBC	DMTL	DMTH
Liver weight (g)	11.81 $\pm$ 0.8	10.66 $\pm$ 1.3	10.51 $\pm$ 1.1	10.33 $\pm$ 1.8
Relative liver weight (%)	3.05 $\pm$ 0.1	3.90 $\pm$ 0.3*	3.72 $\pm$ 0.1*	3.80 $\pm$ 0.3*
Liver glycogen (mg/g tissue)	37.30 $\pm$ 28.9	95.76 $\pm$ 1.3*	91.69 $\pm$ 10.0*	87.02 $\pm$ 21.4*

Values are shown as the mean $\pm$ SD of 7 animals. *p < 0.05 vs. NC (Tukey-Kramer's multiple range *post-hoc* test).

Table 2. Serum data of the different animal groups at the end of the experimental period

Rat groups/ serum parameters	NC	DBC	DMTL	DMTH
Insulin (pmol/L)	65.8 $\pm$ 6.49	8.77 $\pm$ 2.24*	9.31 $\pm$ 2.45*	11.11 $\pm$ 2.98*
Fructosamine (μ mol/L)	223.7 $\pm$ 26.7	242.0 $\pm$ 20.4	246.0 $\pm$ 17.10	233.0 $\pm$ 21.9
AST (U/L)	57.86 $\pm$ 3.9	59.20 $\pm$ 6.3	65.67 $\pm$ 14.7	53.20 $\pm$ 6.1
ALT (U/L)	34.86 $\pm$ 6.4	65.20 $\pm$ 21.7*	53.83 $\pm$ 13.7	62.20 $\pm$ 17.8*
Creatinine (mmol/L)	0.43 $\pm$ 0.1	0.38 $\pm$ 0.1	0.39 $\pm$ 0.1	0.41 $\pm$ 0.1
Total proteins (g/dL)	6.43 $\pm$ 0.4	5.58 $\pm$ 0.2*	4.88 $\pm$ 0.3*#	4.78 $\pm$ 0.2*#
Albumin (g/dL)	2.66 $\pm$ 0.3	2.47 $\pm$ 0.2	2.28 $\pm$ 0.1*	2.25 $\pm$ 0.1*
Uric acid (mg/dL)	2.71 $\pm$ 1.1	1.90 $\pm$ 0.9	1.55 $\pm$ 0.4*	1.76 $\pm$ 0.2

Values are shown as the mean $\pm$ SD of 7 animals. *p < 0.05 vs. NC, #p < 0.05 vs. DBC (Tukey-Kramer's multiple range *post-hoc* test).

concentration. Several critical findings were established, including determining that the selection of leaf area is essential to the level of medicinal potential. Shoots of mulberry leaves contained significantly higher DNJ content than young leaves, which in turn, had significantly higher DNJ content than older mulberry leaves (shoots > young leaves > mature leaves). These findings distinguished better commercial varieties from those with insufficient DNJ concentrations to elicit any beneficial effect on hyperglycemia. Moreover, they tested the quality of the tea-making protocol provided by the manufacturers for DNJ extraction at 90°C for 300 s. Upon modification of the protocol, the optimal tea making condition was in fact found to be at 98°C for 400 s and this increased DNJ extraction from 85 to 95%. This indicates any inferior mulberry leaf selections in tea harvesting and/or coupled with incorrect preparation would most likely provide tea brews containing less the required DNJ concentration to elicit any anti-diabetic effect. A major limitation of many mulberry tea brands is the non-specification of an effective dose to reduce NFBG, despite the packaging claims of anti-hyperglycemic effects of the tea (27). Furthermore, Vichasilp et al. (26) acknowledged the fact that for daily home use it is largely impractical to measure precise tea brewing temperatures for a specified time period in order to ensure sufficient active ingredients are obtained. Although the concentration of DNJ in our brewed mulberry tea has not been measured, no significant anti-diabetic effect might be due to insufficient DNJ concentration.

For this reason, despite much literature indicating the hypoglycemic effects (14, 28–30) of white mulberry leaf tea and extracts, it is most likely that the brand of tea used in this experiment might not provide sufficient DNJ to reduce NFBG as seen in Figures 1 and 2 and might have some other phytochemicals, which did not improve but relatively worsened the glucose tolerance ability as seen in Figure 3. There is, however in fact no record of brewed mulberry leaf tea tested in an animal model for T2D, with most of the studies having dosed the extract itself, or isolating and dosing DNJ into experimental animals to examine the anti-diabetic effects (16, 28, 30, 31). Since mulberry leaf teas are usually consumed after brewing but not as an extract so the aim of our study was to examine anti-diabetic effects of a low (0.25%) and a high (0.5%) concentration of brewed white mulberry leaf tea.

The fructosamine test is another test that is the result of a non-enzymatic reaction between glucose and amino acids in the serum and is an early glyca-

tion end product (32). Fructosamine can thus be used to predict the concentration of advanced glycation end products (AGES) and is an indicator of glycemic control over a 3 weeks or longer period (33). Studies indicate that in diabetic animals, fructosamine concentrations increase compared to normal controls (32). Low serum fructosamine thus indicates a good glycemic control and in the case of intervention trials, the effectiveness of the treatment regime. No significant difference in serum fructosamine and insulin in the mulberry tea consuming groups compared to the DBC group (Table 2) suggests that brewed white mulberry leaf tea have no hypoglycemic or insulinotropic effect but may have some hypolipidemic effects at least in this experimental condition.

Kojima et al. (24) tested the hypolipidemic effects of mulberry leaf extract in healthy non-diabetic human subjects and found no significant differences in serum total cholesterol, HDL- and LDL-cholesterols and triglyceride levels after 6 and 12 weeks, however the hypolipidemic effects of mulberry leaf tea may not be similar between normal and diabetic conditions. In our study, significantly lower total cholesterol and comparatively lower LDL-cholesterol and triglycerides in DMTH group compared to the DBC and DMTL groups suggest the possible hypolipidemic effects of mulberry leaf tea where DNJ might be involved since it has been shown to be effective in decreasing lipid accumulation not only *via* increasing β -oxidation but also by increasing adiponectin levels and activating AMP-activated protein-kinase (AMPK) in isolated rat liver (34).

Although hyperglycemia and hypercholesterolemia are the two major contributing factors to the severity of the diabetic condition, it is necessary to analyze other vital biochemical parameters in order to fully assess the anti-diabetic effects. Mild but chronic increases of these transaminases are also commonly seen in T2D due to hepatic insulin resistance from elevated levels of circulating free fatty acids, which in fact, are toxic to hepatocytes, presumably due to oxidative stress from lipid peroxidation and the recruitment of inflammatory cells (35). However, in our study, the either concentration of mulberry leaf tea could not significantly reduce these liver function enzymes (Table 2). The liver glycogen level was not also affected by the either dose of mulberry leaf tea (Table 1).

In summary, white mulberry leaf tea did not exhibit any hypoglycemic effects, contrary to many literature reports possibly due to the inferior blend of tea selection and hence was unable to correct

minor symptoms of T2D including polyphagia, polydipsia, weight gain and liver glycogen as well as major symptoms including glucose intolerance and insulin deficiency. Additionally, it was significantly increased serum ALT, as well as significantly decreased serum total protein and albumin and serum uric acid – the combined effect thus indicating poorly controlled diabetes and signifying insulin resistance. However, significantly lower serum total cholesterol and markedly and dose dependently lower serum LDL-cholesterol and triglycerides indicate the possible hypolipidemic effects of mulberry leaf tea. In conclusion, white mulberry leaf tea did not display any significantly beneficial anti-diabetic effects but may have some promising hypolipidemic effects at least in this experimental condition and further studies are needed to confirm this effect in humans. Additionally, the effects of the different brands of white mulberry leaf tea may be different due to various factors such as geographical location of tea, age of tea leaves, and processing as well as brewing condition of tea. Hence, the results of this study do not exclude the beneficial effects of the other brands of white mulberry leaf tea which has been proven in other studies.

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Conflict of interest

There is no conflict of interest within this manuscript.

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