

The potential of *Clausena lansium* (Lour.) peel extract consumption on hyperglycemia, hyperlipidemia and augmented oxidative stress in type-2 diabetic model rats

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ABSTRACT

Epidemiological studies have shown that the consumption of some plant components can combat type 2 diabetes mellitus (T2DM) and its complications through various biological properties such as antioxidant activity. The extract of wampee peel (WP) exhibits several medical properties, especially antioxidant effects. We aimed to explore the antihyperlipidemic, hypoglycemic, and antioxidant effects of WP peel extract in an experimental T2DM rat model. T2DM was induced in the experimental groups by a high-fat diet and streptozotocin injection (HFD/STZ). Rats with a fasting blood glucose level above 200 mg/dl were considered diabetic and were separated into six groups (control, diabetic, diabetic treated with WP peel extract at 200, 400, or 600 mg/kg, and diabetic treated with metformin at 250 mg/kg). After 4 weeks of treatment, blood samples were collected for analysis. HFD/STZ-induced rats exhibited hyperglycemia, dyslipidemia, increased HbA1c, and increased lipid peroxidation, but reduced serum antioxidant enzyme activities. However, these changes were attenuated in T2DM rats with the highest dose of WP peel extract. Our results provide strong evidence that WP peel extract has antihyperlipidemic, antidiabetic, and antioxidant properties and thus also suggest that WP peel extract may be useful in developing novel agents for the management of T2DM.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is now recognized as a serious public health problem in the 21st century (Zimmet, 2000). As many as 463 million people were affected by T2DM in 2019 and it is predicted that the number will increase to 700 million (constituting 10.9% of the world population) by 2045 (Saeedi *et al.*, 2019). T2DM is now a main cause of mortality and morbidity and imposes an important financial burden on society.

Several studies have demonstrated that T2DM is associated with dyslipidemia (Chehade *et al.*, 2013; Mehta *et al.*, 2021). Increased serum total cholesterol (TC), low-density lipoproteins (LDLs), triglycerides (TG), and/or a reduction in high-density lipoproteins (HDLs) are also associated with T2DM complications such as diabetic peripheral neuropathy (Al-Ani *et al.*, 2011). Medication for T2DM without undesirable side effects remains a challenging question for medical practitioners, encouraging efforts to find novel treatments or strategies for the prevention and treatment of this morbid disorder.

There is much evidence that oxidative stress plays an important role in the pathogenesis of T2DM by inducing insulin resistance or decreasing insulin secretion, which further aggravates the development and progression of T2DM and its complications (Burgos-Morón *et al.*, 2019). In addition to the currently available pharmaceutical treatments, various medicinal

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herbs, fruits, or plant components have been recommended for T2DM management (Bindu and Narendhirakannan, 2019). There is evidence that bioactive compounds present in fruits, herbs, and various parts of plants can lower blood glucose concentrations and inhibit or prevent oxidation processes, helping to preserve β -cell function by reducing β -cell apoptosis (Rahimi *et al.*, 2005). Therefore, the exploration of management strategies in the form of natural food products with antioxidants and hypoglycemic effects for the treatment of T2DM should be encouraged because they have fewer or no side effects and may be more economic.

The large amount of fruit peel stemming from household kitchen waste and industrial processing is not only economically costly but also can cause environmental problems and need to be utilized or managed (Kumar *et al.*, 2020). Furthermore, several studies have revealed that the peel of fruits such as pomegranate, *Punica granatum*, and *Syzygium cumini* exhibit antidiabetic and antioxidant effects (Salwe *et al.*, 2015; Muttakin and Zulfajri, 2020). Thus, converting fruit peel waste into value-added natural antioxidant products to combat T2DM is an attractive challenge.

Clausena lansium (Lour.) Skeels or wampee peel (WP), a medicinal plant found in southern China and northern Thailand, belongs to the family Rutaceae (Fig. 1). Various parts of WP, such as the leaves, fruits, seeds, and roots, are used as a traditional medicine to prevent or alleviate symptoms including asthma, cough, bronchitis, hepatitis, malaria, dermatitis, and gastrointestinal disorders (Liu *et al.*, 1996; Zhu *et al.*, 2020). WP peel also possesses several biological and pharmacological properties such as antioxidant, antimicrobial, antiobesity, anti-inflammatory, antitumor, antifungal, hepatoprotective, neuroprotective, and antioxidant activities (Adebajo *et al.*, 2009; Liu *et al.*, 2019). In addition, it contains many phytochemically bioactive compounds such as flavonoids, polyphenols, coumarins, carbazole alkaloids, amides, and lactams (Xu *et al.*, 2014). Interestingly, the ethanol extract from the peel of WP was found to have a high flavonoid content (49.73 ± 0.63 mg QE/100 g DW) and phenolic content (530.13 ± 0.07 mg GAE/100 g DW) and exhibited strong scavenging activity toward the 2,2-diphenyl-1-picrylhydrazyl radical ($91.33\% \pm 0.27\%$) (Phachonpai and Tongun, 2021). However, to the best of our knowledge, there is no



Figure 1. *Clausena lansium* (Lour.) Skeels or wampee from Nan province, Thailand.

laboratory evidence of the effects of WP peel extract on oxidative stress in T2DM.

Several studies have proposed that the combination of a high-fat diet (HFD) and a low-dose streptozotocin (STZ) injection induces an animal model for T2DM that closely mirrors the different stages of T2DM in humans (Barrière *et al.*, 2018; Chao *et al.*, 2021). An HFD initiates the insulin resistance that is one of the main pathophysiological features of T2DM (Lackey *et al.*, 2016). At the same time, low-dose STZ has been reported to induce mild impairment of insulin secretion, which mimics the features of the later stages of T2DM (Sahin *et al.*, 2007). Therefore, we investigated the antidiabetic and antihyperlipidemic properties of WP peel extract and its antioxidant effect in an animal model of T2DM induced by an HFD combined with a low-dose STZ injection.

MATERIALS AND METHODS

Drugs and chemicals

STZ, bovine serum albumin, trichloroacetic acid, thiobarbituric acid (TBA), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich (St. Louis, MO). Rat ELISA kits for glutathione peroxidase (GPx) were purchased from Cayman (MI). All other chemical reagents used in this study were of analytical grade.

Wampee peel extract preparation

Mature WP fruits were collected from a local farm in Tanao district, in the central part of Nan province, Thailand. WP peels were removed and washed thoroughly twice under running tap water and dried in a hot air oven at 40°C for 48 hours. WP peel samples were homogenized with an electric blender and were macerated with 70% ethanol and distilled water for 72 hours, vacuum filtered, and evaporated using a rotary evaporator at 40°C. The final dry weight of WP peel extract was used to calculate the percentage yield, resulting in the extraction yield (10.93%). All samples were stored in an amber-colored reagent bottle at 4°C for further analysis.

Animals and experimental protocol

Experiments were conducted in adult male Sprague-Dawley rats, weighing between 220 and 250 g, purchased from Nomura Siam International Co., Ltd. (Bangkok, Thailand). They were placed in stainless steel cages (three rats per cage) under controlled ambient temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$), humidity (55%–65%), and a 12:12-hour light-dark cycle. The rats were fed with standard pellet diet and water *ad libitum* before any food-related treatment began. All the protocols in this study were carried out following the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health (1985) and were approved by the Animal Ethics Committee of the University of Phayao, Thailand (approval number: 5801040006).

After 7 days of acclimatizing to laboratory conditions, all rats were fed an HFD (60% fat, 20% carbohydrates, and 20% protein) except for the uninduced control. Then, 4 weeks later, they received a low dose of STZ (30 mg/kg, intraperitoneally) to induce T2DM and 72 hours later; the fasting blood sugar (FBS) levels of all rats were determined using an Accu-Chek glucometer (Roche Diagnostic, Germany). Only rats with FBS levels ≥ 200 mg/dl

were considered to be T2DM and enrolled in our study. According to the FBS concentration, 60 rats were randomly separated into the following six experimental groups ($n = 10$):

- 1) Normal control group (C): rats were fed on basal pelleted diet and not treated with STZ injection.
- 2) Diabetic control group (T2DM): diabetic rats were fed an HFD initially for 4 weeks before STZ injection. The rats were fed with an HFD for the rest of the experimental period.
- 3) (T2DM + WP peel 200): diabetic rats received WP peel extract in dosages of 200 mg/kg orally once daily for 4 weeks after induction of diabetes. The rats were fed with an HFD for the rest of the experimental period.
- 4) (T2DM + WP peel 400): diabetic rats received WP peel extract in dosages of 400 mg/kg orally once daily for 4 weeks after induction of diabetes. The rats were fed with an HFD for the rest of the experimental period.
- 5) (T2DM + WP peel 600): diabetic rats received WP peel extract in dosages of 600 mg/kg orally once daily for 4 weeks after induction of diabetes. The rats were fed with an HFD for the rest of the experimental period.
- 6) Positive control (T2DM + Met): diabetic rats received metformin in dosages of 500 mg/kg orally once daily for 4 weeks after induction of diabetes. The rats were fed with an HFD for the rest of the experimental period.

Food intake, water consumption, and body weight were recorded weekly. At the end of the experimental period (4 weeks), rats were deprived of food and water for 12 hours, anesthetized with chloral hydrate, and sacrificed by decapitation, and blood was collected to determine HbA1c, lipid profile, lipid peroxidation, and antioxidant enzyme activities.

Determination of glycosylated hemoglobin (HbA1c) and lipid profile

Whole blood was used in the measurement of glycosylated hemoglobin (HbA1c) with the aid of an automatic glycated hemoglobin HbA1c analyzer (HemoCue® HbA1c 501 System, HemoCue AB Co. Ltd., Sweden).

For lipid profile determination (TC; total triglyceride (TG); serum HDL cholesterol); and serum LDL cholesterol), blood was allowed to clot for 15 minutes before centrifuging at 5,000 rpm for 15 minutes to separate the serum. An automatic dry-chemistry analyzer (Fuji Dri-Chem 3,500, Fujifilm, Tokyo,

Japan) was used to determine the amount of TG, TC, and HDL cholesterol. The LDL concentration in serum was calculated according to Friedewald's formula (Friedewald *et al.*, 1972).

Estimation of lipid peroxidation

Serum malondialdehyde (MDA) levels as a marker of lipid peroxidation were determined using a modification of Ohkawa *et al.* (1979) method. The MDA concentration was measured using a Thiobarbituric Acid Reactive Substances assay kit.

Evaluation of antioxidant enzyme activities

Serum superoxide dismutase (SOD) activity was evaluated using the SOD assay kit from Cayman Chemicals according to the manufacturer's instructions. This technique was based on the generation of superoxide anion radicals produced by xanthine/xanthine oxidase. Absorbance of the reaction solution was detected at 505 nm and expressed as U/mg of protein.

Serum GPx activity was determined using a rat GPx ELISA kit (MBS727547, My BioSource, Inc., San Diego, CA), based on the method of Paglia and Valentine (1967). The activity of GPx was recorded spectrophotometrically at 450 nm and expressed as U/mg of protein.

Statistical analysis

All results were reported as mean \pm SD. Statistical analysis of data was conducted by ANOVA followed by the Duncan multiple test using SPSS version 11.5 for Windows (SPSS, Chicago, IL). Differences were judged significant at $p < 0.05$.

RESULTS

The effects of WP peel extract on food consumption and water intake

Our results showed that the T2DM rats had a higher ($p < 0.05$) intake of food and water than that of control rats, which is consistent with the characteristics of T2DM. However, the increase in food and water intake was reversed by a high dose of WP peel extract as well as by metformin (Table 1).

The effect of WP peel extract on body weight

The mean body weight of all treated groups at the end of the experimental period is shown in Figure 2. At the beginning of the experiment, there was no significant difference in the mean body weight between experimental groups. However, it was observed that the mean body weight of the T2DM groups administered WP peel extract at doses of 200 and 400 mg/kg was significantly ($p < 0.05$) higher than that of the control group and showed characteristics of obesity after 1 week of treatment. Oral

Table 1. Effects of WP peel extract on food consumption and water intake in T2DM rats.

Group	Control	T2DM	T2DM + WP peel 200	T2DM + WP peel 400	T2DM + WP peel 600	T2DM + Met 500
Food intake (g/kg BW/day)	28.89 \pm 3.15	35.51 \pm 2.01 ^a	34.62 \pm 4.63 ^a	32.42 \pm 5.02 ^a	23.74 \pm 2.71 ^b	22.62 \pm 3.05 ^b
Water consumption (ml/kg BW/day)	57.13 \pm 4.07	64.70 \pm 7.27 ^a	63.75 \pm 10.71 ^a	61.03 \pm 12.89 ^a	56.37 \pm 5.43 ^b	57.19 \pm 6.04 ^b

T2DM: type 2 diabetic rat; WP: wampee peel extract; Met: Metformin.

^a $p < 0.05$ versus control group, ^b $p < 0.05$ versus T2DM group.

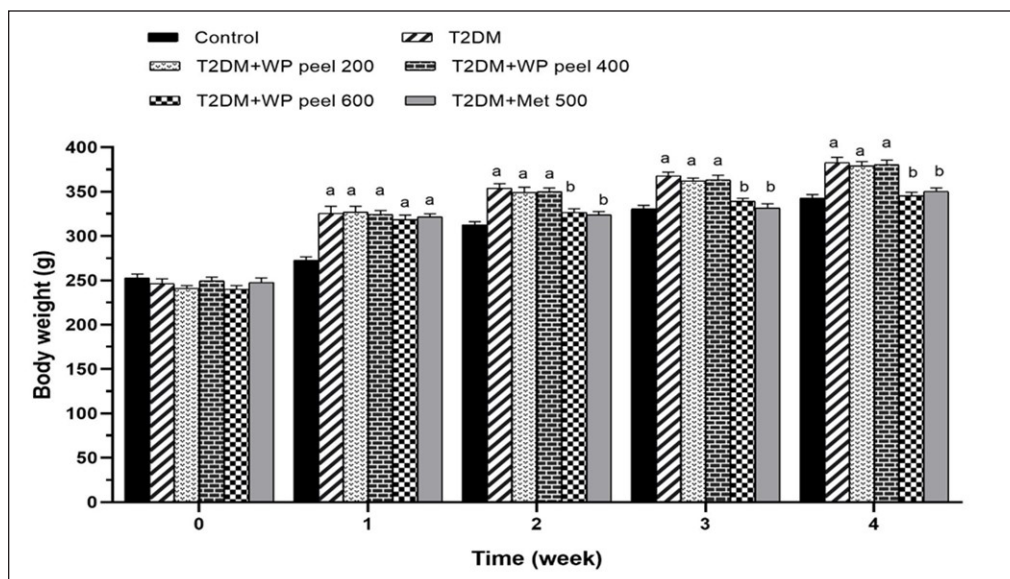


Figure 2. Effect of WP peel extract on body weight in T2DM rats. ^a $p < 0.05$ versus control group, ^b $p < 0.05$ versus T2DM. T2DM: type 2 diabetic rat; WP: wampee peel extract; Met: Metformin.

administration of a high dose of WP was able to reduce body weight when compared with control T2DM rats ($p < 0.05$). Moreover, rats treated with the highest concentration of WP peel extract showed no significant changes in body weight gain when compared with the control group after 2 weeks. This pattern continued throughout the experimental period. The results suggest that a high dose of WP peel extract was able to attenuate weight gain in T2DM rats.

The effect of WP peel extract on FBS concentration

According to the results presented in Figure 3, an HFD- and STZ-induced T2DM in rats, has indicated the significant increase in FBS concentration compared to control rats. Interestingly, rats subjected to WP peel extract at a dose of 600 mg/kg and metformin showed a significantly ($p < 0.05$) lower increase in FBS concentration in the 2nd week of treatment, and this effect continued until the end of the experiment. However, rats administered WP peel extract at doses of 200–400 mg/kg showed no significant change in this parameter.

The effect of WP peel extract on HbA1c and lipid profile

The HbA1c and lipid profile analysis of all rats in the treated groups is presented in Table 2. Our results showed that the rats fed an HFD and administered STZ experienced a significant ($p < 0.05$) increase in HbA1c levels compared to control rats. In addition, the control T2DM rats showed a significant ($p < 0.05$) increase in lipid profile concentrations at all times during the study as compared with all experimental groups. However, a significant ($p < 0.05$) reduction in HbA1c levels was observed in rats treated with both metformin and WP peel extract at a dose of 600 mg/kg compared with the control T2DM rats. Moreover, the one-way ANOVA revealed a significant ($p < 0.05$) reduction in the lipid profile concentrations as compared with the control T2DM rats. Unfortunately, low and medium doses of WP peel extract neither have any effect on HbA1c levels nor were the lipid profile concentrations significantly affected compared with the control T2DM rats.

The effect of WP peel extract on serum antioxidant enzyme activities and lipid peroxidation

Our data showed that rats treated with an HFD and low-dose STZ injection experienced a significant ($p < 0.05$) increase in serum MDA levels but a decrease ($p < 0.05$) in SOD and GPx activities compared to the control group. On the other hand, in the T2DM group that received WP peel extract at a dose of 600 mg/kg, there were significantly ($p < 0.05$) lower MDA levels, but significantly ($p < 0.05$) higher SOD and GPx activities when compared with the control T2DM rats (Table 3).

DISCUSSION

The possibility of developing nutraceutical agents from plants or fruit wastes has been receiving increased attention because of their biological and pharmacological properties that could aid in preventing and treating several diseases including T2DM. Our results indicate that the consumption of WP peel extract was quite effective at lowering blood glucose, HbA1c, lipid profile levels, body weight gain, and oxidative stress in a rat model of T2DM. In addition, treatment with various doses of WP peel extract did not produce any significant adverse effect or changes in behavior or mortality in the experimental rats.

One hallmark symptom of T2DM is chronic hyperglycemia, which results from the impaired metabolism of carbohydrates, lipids, and proteins and leads to uncontrolled lipolysis (Nolan and Prentki, 2019). Extreme hunger and excessive thirst are also related to hyperglycemia (Bibak *et al.*, 2014). Uncontrolled high blood sugar concentrations are associated with the process of glucose autooxidation, protein glycation, and activation of the polyol pathway (Ighodaro, 2018). All these pathways lead to the generation of reactive oxygen species (ROS) and increase lipid peroxidation products in various tissues (Forbes and Cooper, 2013).

Owing to interactions between lipid metabolism and hyperglycemia, glycolipid metabolic disorder is becoming a main factor in T2DM (Perry *et al.*, 2014). Several studies have confirmed

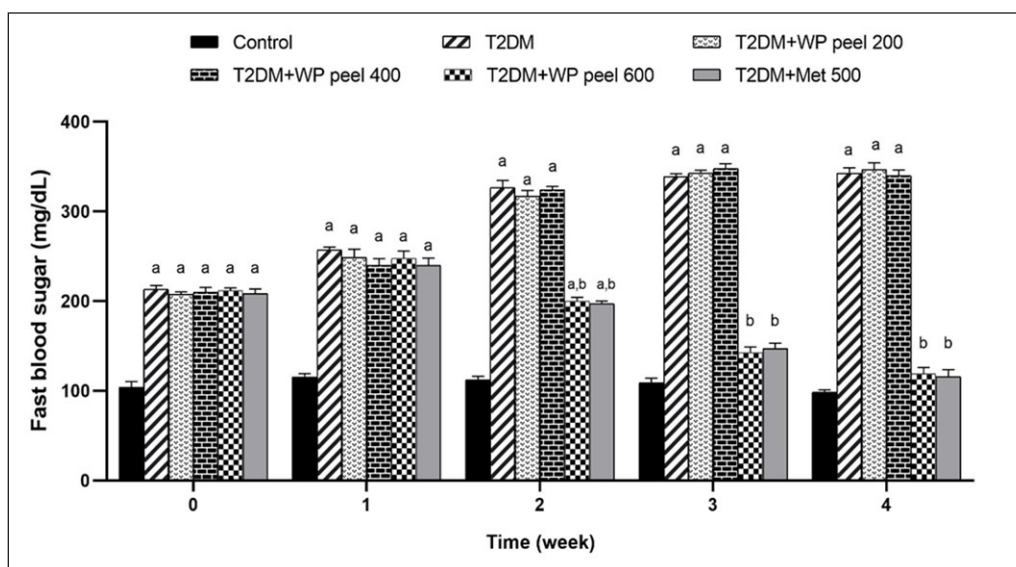


Figure 3. Effect of WP peel extract on fasting blood glucose level in T2DM rats. ^a $p < 0.05$ versus. control group, ^b $p < 0.05$ versus T2DM. T2DM: type 2 diabetic rat; WP: wampee peel extract; Met: Metformin.

Table 2. Effects of WP peel extract on HbA1c and lipid profile in T2DM rats.

Group	HbA1c (g/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
Control	4.82 ± 0.11	89.25 ± 0.83	40.35 ± 0.41	34.16 ± 0.45	73.72 ± 0.03
T2DM	7.89 ± 0.07 ^a	194.33 ± 0.35 ^a	30.12 ± 0.57 ^a	129.60 ± 1.42 ^a	173.05 ± 1.05 ^a
T2DM + WP peel 200	7.02 ± 0.13 ^a	186.87 ± 1.14 ^a	31.29 ± 0.63 ^a	121.65 ± 0.85 ^a	169.88 ± 2.17 ^a
T2DM + WP peel 400	7.24 ± 0.09 ^a	193.24 ± 1.83 ^a	31.77 ± 0.59 ^a	125.12 ± 1.58 ^a	181.75 ± 1.72 ^a
T2DM + WP peel 600	4.67 ± 0.07 ^b	88.61 ± 1.02 ^b	38.24 ± 0.70 ^b	36.31 ± 0.70 ^b	70.32 ± 1.55 ^b
T2DM + Met 500	4.53 ± 0.08 ^b	98.05 ± 0.78 ^b	36.96 ± 0.78 ^b	42.09 ± 0.92 ^b	86.02 ± 2.33 ^b

T2DM: type 2 diabetic rat; WP: wampee peel extract; Met: Metformin; HbA1c: hemoglobin A1c; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol.

^a $p < 0.05$ versus. Control group, ^b $p < 0.05$ versus. T2DM group.

Table 3. Effect of WP peel extract on serum lipid peroxidation and antioxidant enzymes activities in T2DM rats.

Group	MDA (nmol/g protein)	SOD (U/mg protein)	GPx (U/mg protein)
Control	2.70 ± 1.14	4.01 ± 0.37	8.75 ± 1.02
T2DM	7.33 ± 1.36 ^a	1.48 ± 0.31 ^a	3.72 ± 0.94 ^a
T2DM + WP peel 200	7.18 ± 1.40 ^a	1.52 ± 0.52 ^a	3.59 ± 1.75 ^a
T2DM + WP peel 400	6.94 ± 1.53 ^b	1.77 ± 0.34 ^a	4.06 ± 1.70 ^a
T2DM + WP peel 600	5.22 ± 1.07 ^b	4.33 ± 0.21 ^b	7.87 ± 0.78 ^b
T2DM + Met 500	5.14 ± 1.22 ^b	3.90 ± 0.20 ^b	6.80 ± 0.80 ^b

T2DM: type 2 diabetic rat; WP: wampee peel extract; Met: Metformin; MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase.

^a $p < 0.05$ versus. control group, ^b $p < 0.05$ versus. T2DM group.

that a low-dose STZ injection following HFD feeding in a rat model is a good choice for inducing impaired insulin secretion and insulin resistance, similar to the significant characteristic features of the later stages of T2DM (from insulin resistance to β -cell failure) (Gheibi *et al.*, 2017; Srinivasan *et al.*, 2005). A high single dose of STZ is widely used for experiments attempting to cause severe T1DM through direct toxicity to pancreatic islet β -cells (Schnedl *et al.*, 1994). Reed *et al.* (2000) reported that when a low

dose of STZ was used after high-fat feeding, the functions of the β -cell mass and β -cell capacity were moderately impaired without fully compromising insulin secretion, resulting in impaired insulin glucose tolerance. Similar to our study, a rodent model of T2DM was successfully induced with an HFD and a low-dose STZ (30 mg/kg) injection. All the T2DM rats developed signs of diabetes, that is, hyperglycemia, polydipsia, and hyperphagia and as a result, high levels of FBS and HbA1c, increased weight gain,

and excessive thirst and eating. In addition, an increase in plasma levels of TG, TC, and LDL and a low level of HDL were seen in the T2DM rats.

Our work clearly demonstrated that WP peel extract at a dose of 600 mg/kg has an antihyperglycemic effect by reducing FBS concentrations and HbA1c levels in experimental rats with T2DM. It was also observed that the antihyperglycemic effect of the WP peel extract given at a dose of 600 mg/kg was similar to that of metformin, which suggests that WP peel extract might have almost the same potential as metformin, a standard antidiabetic drug for T2DM management (Han *et al.*, 2017).

The increment in food consumption and body weight is thought to be due to a decrease in the activity of endocrine regulators such as insulin and leptin, which mainly act on the hypothalamus, which in turn plays a crucial role in the regulation of energy balance and food intake (Amitani *et al.*, 2013). In addition, excessive fluid intake following an insulin deficiency is promoted by a reduction in the hormones regulating satiety, such as cholecystokinin, peptide YY, and glucagon-like peptide-1, or a decrease in leptin receptor activity (German *et al.*, 2010). However, T2DM rats that were treated with the highest dose of WP peel extract showed a significant attenuation in body weight gain and in the signs of polyphagia and polydipsia, which may be the result of better control of blood glucose level in diabetic rats.

Numerous epidemiological, clinical, and preclinical studies and *in vivo* and *in vitro* experiments have revealed that fruit peels are rich sources of bioactive compounds with antioxidative, antidiabetic, and hypolipidemic properties (Nanasombat *et al.*, 2019; Sun *et al.*, 2020). In our study, the treatment of T2DM rats with WP peel extract at a dose of 600 mg/kg led to a reduction in TC, TG, and LDL levels, suggesting that it may exert antihyperlipidemic effects. However, there was no difference in HDL levels between group receiving the high dose of WP peel extract and the control group, indicating that the WP peel extract may not affect HDL.

Hyperglycemia is a major cause of free radical formation, which can lead to increased lipid peroxidation and inadequate enzymatic antioxidant defense, which further aggravates impaired glucose tolerance in biological systems (Tangvarasittichai, 2015). An imbalance between antioxidant status and oxidation has been proposed to play a pivotal role in mediating insulin resistance (Singh *et al.*, 2022). The primary antioxidant defense system against oxidative stress-induced cell damage consists of three antioxidant enzymes: SOD, CAT, and GSH-Px (Ighodaro and Akinloye, 2018). Usha *et al.* (2017) reported that various antioxidants, vitamins (A, B, C, E, and K), flavonoids, carotenoids, glycerophospholipids, polyphenols, zeaxanthin esters, and phytosterols can reduce oxidative stress and also have an antidiabetic effect (Usha *et al.*, 2017), and our studies indicated the same. The T2DM rat model shows a buildup of oxidative stress due to the reduced activity of SOD and GPx. In addition, our team found that not only did the activities of SOD and GPx decrease in the serum, but also there was an increase in the concentrations of MDA in the T2DM rats. On the other hand, treatment of T2DM rats with WP peel extract at a dose of 600 mg/kg restored the levels of SOD and GPx in the serum. This was accompanied by a decrease in the plasma MDA concentration. Reports have shown that antioxidant supplementation could

protect against the deleterious cellular and biomolecular effects that lead to islet cell dysfunction and subsequent development of T2DM (Karunakaran and Park, 2013). WP peel extract is a complex mixture that contains numerous active ingredients with a unique broad spectrum of pharmacological activities such as flavonoids, polyphenols, lactams, carbazole alkaloids, amides, and clausena coumarine, and in particular, coumarins, which are reported to possess antidiabetic and antioxidant activities (Mukherjee *et al.*, 2006; Prasad *et al.*, 2010). This suggests that the antidiabetic effect mediated by WP peel extract may be due to various bioactive compounds of this crude extract, which also possess the potential for additive, antagonistic, or synergistic activities. Further work is recommended to identify the unknown bioactive compound(s) in WP peel extract that may contribute to its antidiabetic activity.

Unfortunately, the low and medium doses of WP peel extract had no antidiabetic effect. The lack of effect may be related to insufficient concentrations of bioactive compounds in the WP peel extract, which may not have reached the therapeutic or plateau level of treatment. These possibilities will be addressed in the next phase of our research.

In summary, WP peel extract supplementation at a dose of 600 mg/kg attenuated hyperphagia and hyperdipsia, ameliorated hyperglycemia and body weight gain, and possessed hypolipidemic activity in T2DM rats. The reduction in oxidative stress caused by increased SOD and GPx activity could be due to the improvement in glycemia promoted by the WP peel extract. Therefore, the imbalance between the production of ROS and enzymatic antioxidant activity could be controlled in T2DM rats.

CONCLUSION

Our findings show that the crude extract of WP, a waste material generated from the consumption of wampee fruit, contains bioactive compounds that possess significant antidiabetic, antihyperlipidemic, and antioxidant properties in the HFD/STZ-induced T2DM rat model. WP peel extract thus shows promise as a nutraceutical in the management of T2DM.

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LIST OF ABBREVIATIONS

DPPH, 2,2-diphenyl-1-picrylhydrazyl; DW, Dry weight; FBS, Fasting blood sugar; GAE, Gallic acid equivalent; GPx, Glutathione peroxidase; HbA1c, Glycated hemoglobin; HDL, High-density lipoprotein; HFD, High-fat diet; LDL, Low-density lipoprotein; Met, Metformin; QE, Quercetin equivalents; SOD, Superoxide dismutase; STZ, Streptozotocin; T2DM, Type 2 diabetes mellitus; TC, Total cholesterol; TG, Triglyceride; WP, Wampee peel.

AUTHORS' CONTRIBUTIONS

Wathita Phachonpai conceived and designed the study; Wathita Phachonpai, Prathakphong Riyamongkol, and Dej Mann

conducted the experiments; Wathita Phachonpai and Terdthai Tongun analyzed all data; Wathita Phachonpai wrote the paper. All authors have read and approved the manuscript.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest regarding the publication of this paper.

ETHICAL APPROVAL

This study got ethical approval by the Animal Ethics Committee of the University of Phayao, Thailand (Approval Number: 5801040006).

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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