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Anti-hyperglycemic effect and subchronic toxicity of the combined extract from Sattagavata - Mathurameha - Tubpikarn anti-diabetic herbal formulae

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ABSTRACT

Background: The three herbal formulae (Sattagavata, Mathurameha, and Tubpikarn) were prescribed to diabetic patients for a long time, and they included Sattagavata for circulatory system promotion, Mathurameha for diabetics and Tubpikarn for hepatoprotection with less scientific support about the effectiveness and toxicity. The *in vivo* anti-hyperglycemic activity and subchronic toxicity of the combined extract from three anti-diabetic Thai herbal formulae in diabetic rats were performed to prove the anti-diabetic effects and find out the subchronic toxicity of these herbal anti-diabetic formulae on kidney and liver in experimental animals. The antioxidant activity and phytochemical studies were also screened. **Methods:** Type II diabetes was induced in male rats with streptozotocin and nicotinamide. These three anti-diabetic Thai herbal formulae were separately extracted and combined at the ratio of 1:1:1. The combined extract was fed to the diabetic rats at the daily doses of 0.5 and 1.0 g/kg for 30 days. The anti-diabetic drug (glibenclamide) at a dose of 5 mg/kg was given as a positive control group. **Results:** It was found that the 2-hour post-prandial plasma glucose (2 h PPG) levels of 1 g/kg fed the group and the glibenclamide-fed group were decreased compared with control group ($P < 0.025$) on day 15th after feeding. In addition, the 2 h PPG levels and HbA_{1c} values of both treated groups and glibenclamide-fed group were significantly lower than those of control group ($P < 0.01$) on day 30th after feeding. Blood examination at day 30th revealed no difference between the control diabetic group and treated groups. The unchanged parameters included blood urea nitrogen, creatinine, albumin, bilirubin, and cholesterol levels. The liver enzymes, aspartate aminotransferase (AST) and alkaline phosphatase, in the control, 1 g/kg extract-fed group and glibenclamide-fed group were not different. However, the blood AST level was increased in the 0.5 g/kg extract-fed group. From the blood study, it did not show the obvious renal and liver toxicity in an animal model after 30 days extract feeding. Phytochemical studies revealed the presence of rutin in Sattagavata formula and chlorogenic acid in Tubpikarn formula which supported the antioxidant effect of these herbal medicines. **Conclusion:** This study indicated that this these combined Thai anti-diabetic herbal formulae had the anti-hyperglycemic action in Type II diabetic animal model and also had the antioxidant action *in vitro*. No obvious renal and liver toxicity were found after feeding for 30 days.

INTRODUCTION

Diabetes mellitus is a major endocrine disorder, especially Type II diabetes occurring in adults throughout the world. The prevalence of diabetes is estimated to reach 330 million by the year 2025, according to International Diabetes Federation, with the great potential increase being in Africa and Asia.[1] According to modern medicine, the Type II diabetic treatments are based on lowering of blood sugar and HbA_{1c}. The drugs were developed based on the mechanisms,

e.g. stimulating β-cell insulin secretion, reducing carbohydrate absorption, increasing glucose uptake in muscle and adipose tissue, exertion of antioxidant effects, and decreasing β-cell apoptosis[2,3] and most of the studies were investigated using a single agent. Nowadays available remedies for diabetes include insulin and many oral anti-diabetic drugs such as sulfonylureas, meglitinides, and biguanides; this medicine could give side effects after prolonged use. From plant sources, more than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new anti-diabetic drugs from natural plants are still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus.[4,5] Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, and carotenoids that are frequently implicated as having anti-diabetic effects.[4] However, the usage of the herbal formula with composed of 5–20 plants is more accepted than the usage of the single plant because of the concept of the multi-target drugs which belief in the synergistic effects of an herbal formula to promote health and relief some disease symptom.[6]

Thai Traditional Medicine (TTM) has many different concepts in human physiology and different paradigm of diabetic treatment.[7] TTM has the same physiological system as Ayurvedic medicine which describes the function of the human body by the Tridosha system, i.e. Vata (wind), Pitta (fire), and Arpo (Shlesma or water and earth). Diabetes in the context of TTM is the failure of Arpo system which affects the Vata system and also the Pitta system. The licensed traditional doctors formulated three herbal formulae based on the TTM theories; these are Mathurameha which directly work on the Arpo system, Sattagavata for the Vata system and Tubpikarn for Pitta system which focuses for the hepatoprotective effect. These three formulas have been prescribed to patients for several decades, especially Mathurameha formula for diabetic patients.[7,8]

These three formulae (Sattagavata, Mathurameha, and Tubpikarn; Table 1) have been prescribed to diabetic patients by a famous traditional doctor, Suraphan Sirithamwanich, for more than three decades. The patients received 1000 mg 3–4 times a day until the blood sugar and HbA_{1C} were normalized and stable. The traditional doctor claimed that after continuous treatment for 1–2 years, most of the patients resumed to the normal stage and able to stop the treatment (Suraphan Sirithamwanich, Personal Communication, 2009).[9] Traditional practitioners and users of medicinal plants believe that herbs and herb extracts are safe simply because they are natural in origin. However, their general acceptance of these formulae has been limited by lack of data on their anti-hyperglycemic action and toxicity.

Some scientific evidences of plant components of these formulae on anti-diabetic effect were reported. The alcoholic extract from the fruits of *Solanum trilobatum* also showed anti-hyperglycemic activity in experimental rabbits in equivalent to that of tolbutamide and also in rats.[10] *Cinnamomum* spp. enhances better utilization of insulin by the human body, as a result, the cells can exploit glucose for their energy and was concluded that it is suitable as an anti-diabetic herb.[11] *Cinnamomum* spp. promotes the insulin sensitivity in human and be useful in human type II diabetes[12] and in rats.[13] Benjagul is one of a combination of herbs called "Pigad;" it comprises five plants which will normalize the four elements according to TTM theory, i.e. Earth, Water, Wind, Fire, and Akash (Air). These plants include *Piper longum*,

Table 1: Main plant composition in the Sattagavata formula, Mathurameha formula, and Tubpikarn formula

Sattagavata formula (remedy for circulatory system promotion)	Mathurameha formula (remedy for diabetics)	Tubpikarn formula (remedy for hepatoprotection)
Dendrophthoe pentandra, Loranthaceae	Pepper (*Piper nigrum*, Piperaceae)	Neem (*Azadirachta indica*, Meliaceae)
Siam Benzoin (*Styrax benzoin*, Styracaceae)	Asiatic pennywort (*Centella asiatica*, Apiaceae)	Black cumin (*Nigella sativa,* Apiaceae)
Eagle Wood (*Aquilaria crassana*, Thymelaeaceae)	Thai nightshade (*Solanum trilobatum,* Solanaceae)	Common cress (*Lepidium sativum,* Brassicaceae)
Nutmeg (*Myristica fragrans*, Myristicaceae)	*Lobelia alsinoides*, Campanulaceae	Cumin (*Cuminum cyminum*, Apiaceae)
Senna (*Senna alexandrina*, Fabaceae)	Turmeric (*Curcuma longa*, Zingiberaceae)	Dill (*Anethum graveolens*, Apiaceae)
Wild Mango Root, (*Mangifera pentandra*, Anacardiaceae)	Sabah Snake grass (Clinacanthus nutans, Acanthaceae)	Anise (*Pimpinella anisum,* Apiaceae)
Saffron (*Crocus sativus*, Iridaceae)	*Dalbergia candenatensis*, Fabaceae	Asiatic pennywort (*Centella asiatica*, Apiaceae)
Red Sugar cane (*Saccharum officinarum*, Poaceae)	*Brucea javanica*, Simaroubaceae	Sabah Snake grass (*Clinacanthus nutans*, Acanthaceae)
Cinnamon (*Cinnamomum cassia*, Lauraceae)	Anatto tree (*Bixa orellana*, Bixaceae)	False Daisy (*Eclipta prostate*, Asteraceae), Five Kots
Camphor Laurel (*C. Camphora*, Lauraceae)	Duck's eyes (*Ardisia elliptica*, Myrsinaceae)	And Benjagul
Gum Asafetida (*Ferula assa-foetida*, Apiaceae)	Pineapple, (*Ananas comosus*, Bromeliaceae)	
And others	And Benjagul	

Piper sarmentosum, *Piper retrofractum*, *Plumbago indica,* and *Zingiber officinale*, respectively.[14] All plants showed beneficial aspects to diabetic conditions. *P. sarmentosum* has been shown that the aqueous extract was able to reduce blood sugar in induced diabetic rabbits but not in normal rabbits.[15] It also significantly reduced blood sugar in induced diabetic type I rats but not in normal rats.[16] However, the *P. sarmentosum* extract could not normalize the plasma glucose level. *P. longum* also has been shown to have anti-diabetic and antihyperlipidemic activities in the streptozotocin (STZ)-induced diabetic rats.[17] The aims of this study are to prove the anti-hyperglycemic effects and find out subchronic toxicity of these herbal anti-diabetic formulae on kidney and liver in experimental animals. Thin-layer chromatography (TLC) was also developed for the quality control purpose. The antioxidant activity was also screened.

MATERIALS AND METHODS

Chemicals

Nicotinamide (NA), o-dianisidine, glucose oxidase, and peroxidase enzyme (PGO) and STZ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Glibenclamide tablets (Daonil) were purchased from Aventis Pharma Ltd (Bangkok, Thailand). All other chemicals and reagents were analytical grade.

Plant materials

All the plant materials of these three formulae from Table 1 were identified and extracted by traditional doctors Ratha bol Sirithamwanich and Thadchawan Sirithamwanich. Briefly, 6 kg of each herbal formula; Sattagavata, Mathurameha, and Tubpikarn were separately extracted by decoction and lyophilized to dryness yielding 220, 200, and 200 g, respectively. All extracts were kept in a closed container at 4°C until used. The lyophilized extracts were freshly combined at the ratio of 1:1:1 for the further study in the diabetic animal model.

Animal and maintenance

Male Sprague-Dawley rats, weighing 120–150 g, were purchased from the National Laboratory Animal Centre (NLAC), Mahidol University, Thailand. They were housed in an air-conditioned room (22–25°C) and subjected to a 12-h light/dark cycle for at least 1 week before the experiment. The animals were given free access to a pellet diet and water *ad libitum*. The protocol of animal experimentation was approved by the Committee of the Animal Care and Use, Faculty of Pharmacy, Mahidol University (PYR 003/2556).

Induction of experimental diabetes

Experimental type II diabetes was induced in rats by STZ (50 mg/kg i.v.) at 15 min after NA (100 mg/kg i.p.) in overnight fasted male rats using the method of Masiello *et al*., 1998[18] with a slight modification. Urine glucose levels were weekly checked by urine glucose strip. Three weeks after the induction, 6-h fasting plasma glucose (FPG) levels were examined, and the rats with FPG level >160 mg/dl were used as diabetic rats. STZ-NA induced diabetic rats were divided into four experimental groups with 7 rats per group. Each group was daily oral administered through the rat feeding tubes with distilled water, two doses of the combined extract dissolved in water, glibenclamide (a reference hypoglycemic drug) for 30 days as follow:

- Group 1: Distilled water (diabetic control)
- Group 2: Combined extract 0.5 g/kg b.w.
- Group 3: Combined extract 1.0 g/kg b.w.
- Group 4: Glibenclamide 5 mg/kg b.w.

Two hours post-prandial plasma glucose (2 h-PPG) levels were determined on day 0 (the day before oral administration started), 15, and 30. Blood samples were collected from tail vein after 2 h fasting and examined for plasma glucose level by glucose oxidase method. Briefly, add 5 ml of the glucose oxidase and peroxidase reagent to 10 μl of rat plasma or water or standard glucose 100 md/dl, mix well and incubate 30 min at 37°C and read the extinction at 450 nm against the reagent blank. The rats were weighed once a week to adjust the dose of treatments.

At the end of the experiment on day 30, HbA_{1C} and full blood chemistry analysis were performed at the blood lab, Siriraj Hosptal, Mahidol University. These were blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin, bilirubin, and lipid profiles by Automate Analyzer at the blood laboratory, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Analysis of chemical compounds

TLC chromatogram was developed using EtOAc:H_2O:formic acid:acetic acid (100:26:11:11) system and using rutin and chlorogenic acid as references.

Antioxidant screening

The detection was done by spraying the TLC chromatogram with natural products-polyethylene reagent (NP/PEG) for flavonoid presence and DPPH for the antioxidant test. DPPH is a stable free radical which possesses a deep purple color and a strong absorption around 517 nm. The antioxidant compounds present in the TLC plate convert DPPH radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom. The color change from the purple of DPPH radical to pale yellow of reduced form of DPPH indicates the antioxidant activity.[19]

Statistical analysis

The data were statistically analyzed using the Student's *t*-test method. $P < 0.05$ was taken as significant.

RESULTS

Effect on the PPG levels of type II diabetic rats

At the beginning of the experiment (day 0), the PPG (2 h-PPG) concentration of the diabetic rats was not different in all groups (350–420 mg/dl) and was significantly higher than the normal rats (60–90 mg/dl).

At day 15th after feeding, the groups treated with an anti-diabetic drug (glibenclamide) and the herbal extract at dose 1 g/kg showed significantly lower PPG levels than control diabetic group. The decreased percentage of PPG levels in 1 g/kg and glibenclamide (5 mg/kg) was 10.7 and 11.4% from the control level, respectively. There was no difference among diabetic groups treated with glibenclamide and 1 g/kg herbal extract treated group.

At the end of the experiment, day 30th, the PPG levels in the glibenclamide treated group and the extract treated groups at doses 0.5 and 1 g/kg, were significantly decreased from the control diabetic group. The decreased percentage of PPG levels in 0.5, 1 g/kg and glibenclamide (5 mg/kg) was 16.9, 11.8, and 15.3% from the control level, respectively. There was no difference among diabetic rats treated with glibenclamide and herbal extracts [Figure 1].

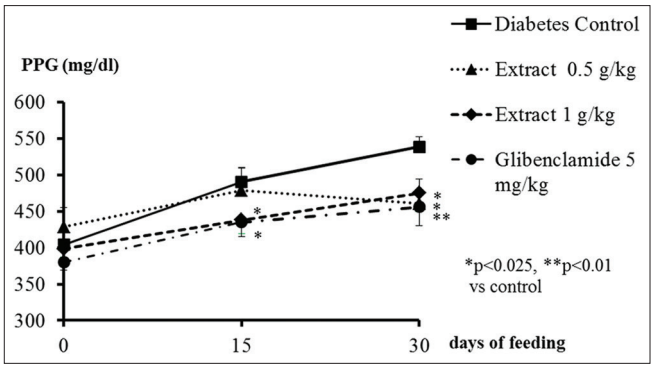

Figure 1: Effect of the combined extract from Sattagavata - Mathurameha - Tubpikarn anti-diabetic herbal formulae on the 2 h post-prandial plasma glucose levels of type II diabetic rats (n = 7)

Effects of herbal extracts on HbA_{1C}

The HbA_{1C} levels of normal rats were 3.3% ± 0.0%. In diabetic rats, HbA_{1C} level of the control untreated group was increased about 3 times. At day 30 after feeding, the HbA_{1C} levels of diabetic rats treated with glibenclamide and herbal extracts (0.5 and 1 g/kg) were 8.3 ± 0.4, 8.3 ± 0.3, and 8.6 ± 0.2%, respectively, and were significantly decreased from control diabetic group (9.1 ± 0.1%). There was no difference among diabetic groups treated with glibenclamide and herbal extracts treated group [Figure 2].

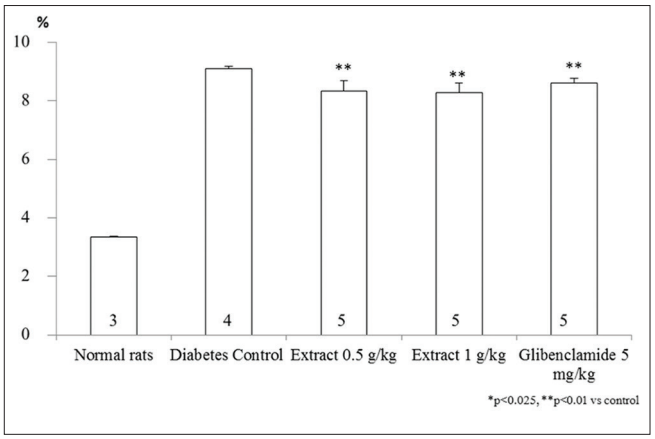

Figure 2: Effect of the Sattagavata – Mathurameha - Tubpikarn anti-diabetic herbal formulae extract on HbA1C (%) of type II diabetic rats. Numbers in bars represent numbers of rats in each group

Subchronic toxicity study

At the end of the experiment, the blood was collected from each experimental rat, and blood chemistry parameters were measured. These parameters included BUN, creatinine, albumin, bilirubin, and triglyceride levels. The BUN and creatinine values did not show different kidney function in all diabetic groups [Table 2]. The liver enzymes, AST and ALP, in the control diabetic group, 1 g/kg extract-fed group, and glibenclamide-fed group were not different. The values of ALT, albumin, and bilirubin were not different from the control diabetic group. However, the blood AST level in the 0.5 g/kg extract-fed group was increased in comparison with the control diabetic group but was not different from other groups. Interestingly, the blood ALP was significantly decreased in the 0.5 g/kg extract-fed group [Table 2].

There was a slightly decrease in cholesterol and triglyceride level in 0.5 g/kg fed rats. There was an increase in cholesterol level in both glibenclamide and 1 g/kg fed rats in comparison with control diabetic group. The significant difference was found in 1 g/kg fed group; however, the cholesterol values were in normal range (40–130 mg/dl). The triglyceride levels were higher than normal range (26–145 mg/dl) but were not significantly different among studied groups [Table 2].

Chemical constituents

The developed TLC system can be used for quality control purpose [Figure 3a]. TLC studies revealed the presence of rutin in Sattagavata formula [Figure 3b], and chlorogenic acid in Tubpikarn formula [Figure 3c], but the total amounts of these two compounds in the formulae were not measured. Furthermore, it was also found that all three herbal formulae had the antioxidant property by showing a positive reaction with DPPH spray [Figure 3a].

DISCUSSION

The Thai Traditional doctor formulated the anti-diabetic formula "Mathurameha" according to "rasa" or the taste of the herb which is bitter. The theory of TTM stated that the bitter taste improves the metabolism, combat the heat, quench the thirst, and correct the urine. Diabetic diabetes mellitus has been recognized since ancient time as "Prameha," and many herbs and formula were given.[14] However, the combination of herbs in "Mathurameha" was unique and surprisingly, the anti-diabetic activity of the major plants was supported by other studies. These herbs are, for example; *Centella asiatica* showed both hypoglycemic and hypolipidemic effects, and rutin was one of the active compounds,[20] the anti-hyperglycemic activity was partly due to inhibition of carbohydrate digestion and glucose-fiber binding.[21] The leaves of *Piper nigrum* had hypoglycemic effect on alloxan-induced diabetic rats.[22] The seeds of *Brucea javanica* had been shown that it possessed anti-diabetic and antioxidant properties.[23] The hypoglycemic effect was due partly to quassinoids,[24] it also inhibits rat intestinal alpha-glucosidase enzymes both sucrase and maltase.[25] The seeds of *Bixa orellana* also showed hypoglycemic

Table 2: Parameters of blood chemistry and blood lipids in diabetic rats after 30 days feeding Sattagavata – Mathurameha - Tubpikarn anti-diabetic herbal formulae (n=7)

Groups or Normal Range	BUN	Creatinine	AST	ALT	ALP	Albumin	Bilirubin	Cholesterol	Triglyceride
Diabetes control	30.6±1.9	0.5±0.0	96.6±10.0	65.1±10.5	697.0±52.7	2.9±0.1	0.3±0.0	71.3±4.9	180.7±25.8
Extracts 0.5 g/kg	36.1±3.0	0.6±0.0	117.1±5.6#	85.7±8.1	344.7±163.1#	3.0±0.1	0.3±0.0	67.7±1.8	158.9±49.2
Extracts 1.0 g/kg	32.9±2.7	0.5±0.0	105.0±9.6	74.1±12.5	626±168.8	3.0±0.1	0.3±0.0	83.0±4.9#	245.7±71.5
Glibenclamide 5 mg/kg	31.0±2.5	0.5±0.0	119.6±23.3	70.6±9.1	571.3±229.5	2.9±0.1	0.3±0.0	74.3±4.3	165.0±44.2
Range in normal rats	15–21 mg/dl	0.2–0.8 mg/dl	45.7–80.8 U/l	56.8–128 U/l	17.5–30.2 U/l	3.8–4.8 g/dl	0.2–0.55 mg/dl	40–130 mg/dl	26–145 mg/dl

#P<0.05 versus control

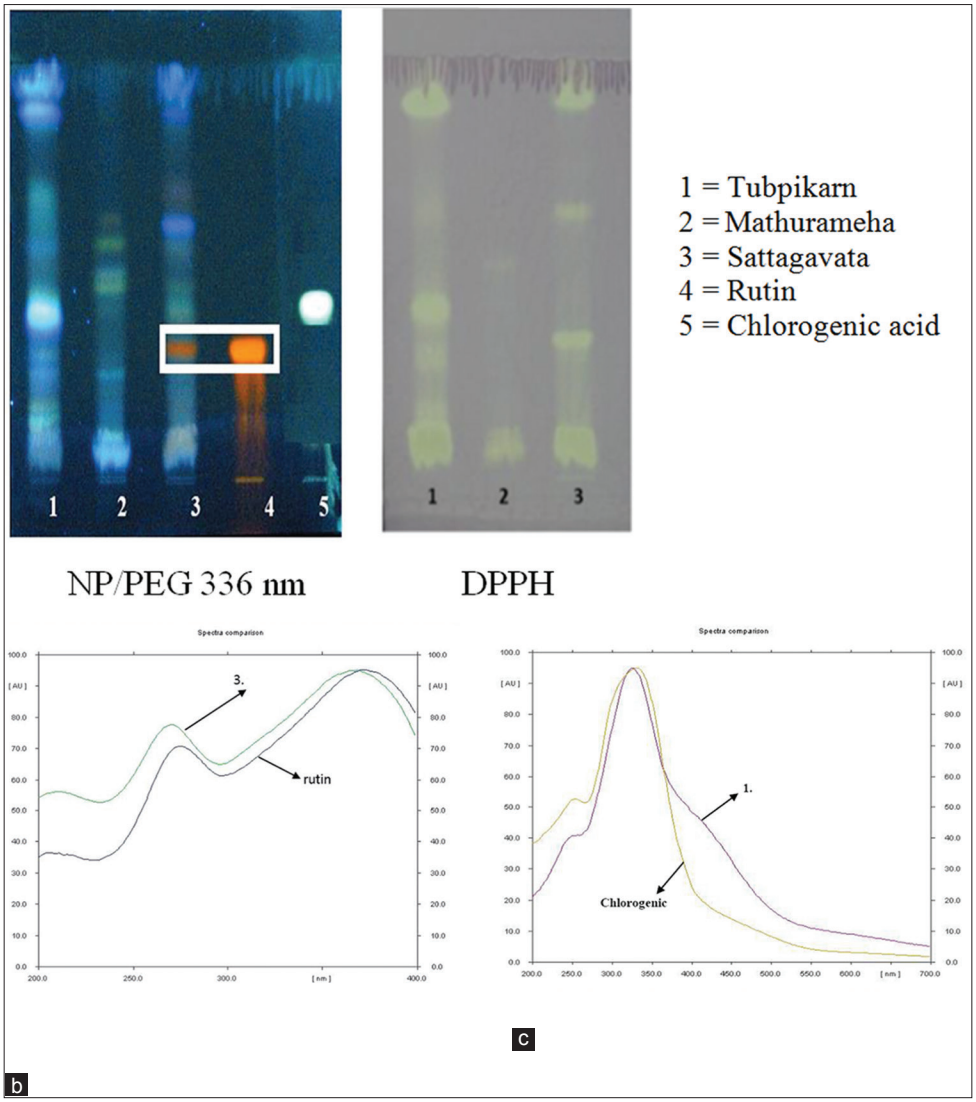

Figure 3: Thin layer chromatography (TLC) fingerprint and ultraviolet scan of the anti-diabetic herbal formulae. (a) TLC system:EtOAc:H_2O:formic acid:acetic acid (100:26:11:11). (b) UV scan of Sattagavata. (c) UV scan of Tubpikarn

activity in dog.[26] Since many formulations contain multiple extracts and compounds, each herbal preparation may contain multiple mechanisms. The proposed mechanism of action of these extracts came from the proved action of herbal ingredients and known compounds are decreasing carbohydrate absorption (rutin's action),[27,28] improving insulin sensitivity (chlorogenic acid's action),[29-32] increasing peripheral glucose uptake (rutin's action),[27,28] and exerting antioxidant effects.

Due to difficulties in human research, animal models of diabetes are useful research tools for this purpose and rodent models of type II diabetic model were the first choice, especially the STZ-NA-induced rat model.[33] STZ-NA induces mildly diabetic rats, which exhibit a mild decline in glucose tolerance due to loss of early-phase insulin secretion, are sensitive to the hypoglycemic effects of insulinotropic agents and have many pathological features resembling type II diabetic, which may be useful in the pharmacological investigation of numerous anti-diabetic drugs.[34]

In the management of diabetes, healthcare providers usually assess glycemic control with FPG as well as by measuring HbA_{1C}. In this study, we designed to measure the PPG concentrations every 2 weeks and at the end of the study to see the effectiveness of treatment. It was shown that the extract could effectively reduce the PPG concentrations [Figure 1]. In diabetes both human and rats, HbA_{1C} is clinical use as a diagnostic marker and helps to indicate the degree of protein glycation, long-term blood glucose and correlation of diabetes associated complication.[35,36] The HbA_{1C} result can be used to estimate the average blood sugar level in the past 2–3 months. In this study, the diabetic rats showed the significant increase of HbA_{1C} levels in comparison with the normal rats [Figure 2]. The extract-treated diabetic rats had a significant decrease in HbA_{1C} levels that could be due to an improvement in glycemic status. The decrease in PPG and HbA_{1C} levels indicated the effectiveness in controlling the hyperglycemic condition in diabetes. Although the extracted-treated group could lower the PPG and HbA1C of the extract-treated diabetic rats, the extract could not lower them to a normal level, there might be the severity of the diabetes condition. In this study, the diabetic condition was quite severe, and the diabetic severity was progressive increase. The anti-hyperglycemic activity of the extract in the severe diabetic condition was acceptable. Even the anti-hyperglycemic drug, glibenclamide, also could also lower but could not normalize the PPG and HbA1C levels [Figure 1].

One main kidney function is to excrete the wastes such as urea, creatinine, and uric acid from the body. The elevated levels of these wastes indicate the malfunction of kidney. After the oral feeding of the herbal extract for the 30 days, the BUN and serum creatinine levels of all diabetic groups in this study were not different. This finding indicated that the three herbal formulae had no sub-chronic toxicity in diabetic rats [Table 2].

Damage to structural integrity of the liver is reflected by an increase in the activity of ALP, probably as a result of leakage from altered cell membrane structure. Therefore, the increase in serum ALP activity in the untreated diabetic rats about 20 times above the normal range [Table 2] confirmed damage to liver plasma membrane. In this study, level of ALP in all the extract-treated diabetic rats showed a tendency to decrease, especially in the dose 0.5 g/kg. The transaminases (AST and ALT) are well-known enzymes used as biomarkers to predict possible toxicity to liver.[37] These transaminases increased in the untreated diabetic rats about 2 times above the normal range [Table 2]. These transaminases were not different among groups of study except the dose of 0.5 g/kg, AST was significantly increased from the control diabetic group, but at a higher dose it did not differ from the control diabetic group [Table 2]. Albumin is a protein molecule that used to assess the health status of the liver. Albumin is created by the liver. Reduction of albumin was found in all diabetic groups, and there were no differences among all diabetic groups. Bilirubin is the major product that results from the breakdown and destruction of old red blood cells and bilirubin is removed from the body by the liver. In this study, the bilirubin levels are in normal levels in all groups. From all blood chemistry in Table 2, we concluded that the three herbal formulae had no obvious sub-chronic toxicity on liver and kidney in diabetic rats. Thus, we also indicated that the oral use of the three herbal formulae had no obvious toxicity on main vital organ liver and kidney even in diabetic condition within 1 month in an animal model.

The results showed effectiveness of the three herbal formulae at the dose of 0.5–1 g/kg in rat equivalent to 5–10 g/day human patients 60 kg weight (calculated the human equivalent dose or HED based on body surface area by multiply the animal dose by 0.162)[38] which support the total daily dose of 9–18 g/day of the herbal formula. These formulae for the treatment of Type II diabetic were as safe as glibenclamide on the liver and kidney.

Abnormalities in lipid profile are very common in the diabetic state,[39] and the most common are hypertriglyceridemia and hypercholesterolemia.[40] In this study, we found an increase in cholesterol levels only in the 0.5 g/kg extract-treated group, but this cholesterol levels were in the normal range [Table 2]. The triglyceride levels were increased above the normal value and were not different among a group of study.

In this study, the use of combination 3 TTM (Sattagavata, Mathurameha, and Tubpikarn) was tested to prove the benefit for a holistic approach. The results indicated the effectiveness of anti-hyperglycemic or anti-diabetic effects from the reduction of PPG and HbA1c. However, the combination of 3 TTM did not significantly improve other clinical biochemistry value especially liver function values (AST ALP ALT). These results might not prove the benefit for a holistic approach, only the anti-hyperglycemic activity and the subchronic toxicity were tested. The restoration of the liver function in severe diabetes may need a higher dosage or a longer period of treatment of the Tubpikarn formula. The experiment about the effectiveness and dosage of Tubpikarn formula and the active fraction or compounds of the three herbal formulae should be done in future.

The TLC chromatogram was developed using EtOAc:H_2O:formic acid:acetic acid (100:26: 11:11) mixture, the detection was done by spraying with NP/PEG for flavonoids and DPPH for antioxidant activity [Figure 3]. It was found that all three herbal formulae contained some antioxidant activity. Tubpikarn formula appeared to have higher antioxidant activity than the other two formulae; these could be partly due to the presence of chlorogenic acid. Sattagavata formula also showed the presence of flavonoid rutin, the flavonoid which showed beneficial effects on blood vessels and circulation.[41,42]

CONCLUSION

This study indicated that these combined Thai anti-diabetic herbal formulas had anti-hyperglycemic activity in STZ-NA

induced diabetic rats which could be the animal model of Type II diabetes; these herbal formulas have a unique combination. This was proved by the hypoglycemic effect and the reduction of HbA_{1C}. The study also showed no obvious sub-chronic toxicity on liver and kidney function which was not different from glibenclamide or control diabetes. TLC examination also showed the presence of rutin and chlorogenic acid which are powerful antioxidants and are beneficial to diabetic conditions.

The appropriate dose appeared to be 1 g/kg in rats or 10 g/day for 60 kg human patients. The fact that TTM has several herbs used for Type II diabetes demonstrates that there are many herbs which can be utilized for the treatment. It is the big opportunity for both traditional and modern medicine to explore anti-diabetic drug which is no obvious sub-chronic toxicity on liver and kidney function.

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