

NATURAL DRUGS

PRELIMINARY INVESTIGATION OF NORMOGLYCEMIC,
ANTI-HYPERGLYCEMIC AND DYSLIPIDEMIC ACTIVITIES OF DIFFERENT
EXTRACTS OF *TINOSPORA CRISPA* ON DIABETIC RATMIR MOHAMMAD REZA SEYED HASSANI¹, ASHFAQ AHMAD¹, MOHD. ZAINI ASMAWI^{1*}
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Abstract: *Tinospora crispa* is an indigenous medicinal plant belonging to Menispermaceae family, known locally as “akar patawali” or as “akar seruntum”. Different parts of this plant are used for various therapeutic purposes for treatment of hypertension, stimulation of appetite, protection from mosquito bites and to treat ailments like jaundice, wounds, intestinal worms and skin infections, tooth and stomach aches, coughs, asthma and pleurisy. The stems were washed, dried, pulverized using Wiley Laboratory Mill apparatus and stored in dry air-tight plastic containers until use. Then, they were extracted successively with petroleum ether, chloroform, methanol and finally with water by maceration. The solvent was evaporated from each extract (using rotary evaporator), dried extract were stored at -4°C until used. Animals were randomly divided into six groups and each group having 6 animals for two sets of experiments. In the first test, healthy rats were treated orally with the plant extracts (1 g/kg) suspended in Tween 80. In the second test, the diabetic rats were fasted overnight. The test groups of 6 diabetic rats were treated orally with extracts of *Tinospora crispa* (1 g/kg), glibenclamide (10 mg/kg) and 10% Tween 80, (10 mL/kg), respectively. In these results, there was no significant effect on blood glucose levels in normal rats treated with different extracts of *Tinospora crispa*. The water extract of *Tinospora crispa* decreased the glucose levels in diabetic rats and has the ability to reduce lipid profile in diabetic rats as compared to control diabetic rats.

Keywords: *Tinospora crispa*, glibenclamide, diabetic, dyslipidemic

Diabetes mellitus (DM) as a major public health problem, has increased prevalence in the modern world (1). It is a group of metabolic diseases characterized by chronic high blood sugar, either because the body does not produce enough insulin, or that target cells do not respond to the insulin that is produced (2). The high blood sugar produces the classical symptoms such as polyuria, polydipsia (increased thirst) and polyphagia (increased hunger). The World Health Organization estimated a total of 171 million people with DM from the global population in 2000, and it has been projected to increase to 366 million by 2030 (3). Available research findings indicate that type 2 diabetes is no longer a disease of middle or old age, rather it now affects children of 10 years old or above, hence necessitating a holistic approach in looking for a cure.

Along with pharmacological treatment, non-pharmacological treatment has become essential for

the prevention and management of diabetes mellitus at such related conditions including malnutrition and cardiovascular diseases. For instance, dietary advice given to older people with diabetes is intended to maintain adequate nutrition thereby complementing the observed tissue wasting. However, besides the nutritional complementary role, most leafy vegetable also exert therapeutic functions, in which case they are referred to as functional foods. Natural products in the medicinal plants have been used as a source of drugs in the traditional medicine (4, 5).

Tinospora crispa is one such vegetable with nutritional and pharmacological functions. *Tinospora crispa* is an indigenous medicinal plant belonging to Menispermaceae family, known with local name “akar patawali” and “akar seruntum”. It is an indigenous medicinal plant that commonly grows wild in Asian countries including Malaysia Indonesia, Thailand and the Philippines (6). The

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leaves, roots, stems, all have their own miracle in curing diseases (7) that has a folk reputation in rural southern India as a hypoglycemic agent. For many years, *Tinospora crispa* has also been used in South East Asia especially India, China and Malaysia for treating various ailments such as prurigo, eczema, impetigo and oxidative stress conditions (8), Whole *T. crispa* is used to treat cholera among the Malay community and its stem for various therapeutic purposes such as treatment for hypertension, stimulation of appetite and protection from mosquito bites and to treat ailments like jaundice, wounds, intestinal worms and skin infections, tooth and stomach aches, coughs, asthma and pleurisy (9), and in Thai a decoction from the stems has been used as an antipyretic, for treating internal inflammations, reducing thirst, cooling down the body temperature and for maintaining good health (10). Sometimes, it was also used as an anti-parasitic agent in both man and domestic animals (6, 11) This plant, able to cause a reduction in serum glucose level in diabetic rats, and the hypoglycemic effect, was probably due to its insulinotropic activity also increased peripheral utilization of glucose and inhibited hepatic glucose release (1). *T. crispa* has been demonstrated to possess antifilarial, antimalarial, antipyretic, antibacterial, and antihyperglycemic effects (6). In Indonesia (Borneo) it has been used to treat lumbago (10).

In another study, *Tinospora crispa* ethanol extract was not capable of lowering blood sugar and insulin levels (12). Previous studies on this herb showed that its aqueous extract was able to cause a reduction in blood glucose level in moderately diabetic rats, and the hypoglycemic effect was probably due to its insulinotropic activity (13).

Malaysian diabetics are also at high risk of macro vascular complications including ischemic heart disease and stroke, in part due to late diagnosis and poor glycemic control but also because of the high association with other components of the metabolic syndrome such as obesity, hypertension (10-37%) and hyperlipidemia (63-76%) (14). Hence, the dare for finding a new method such as a utilizing herb/s in treating and controlling diabetes. In South East Asian countries like Malaysia and China, an aqueous extract of *Tinospora crispa* is taken orally as a diabetes treatment. The Malays in Malaysia consider the vine as a universal medicine and its the most popular local medicinal plants. Makabühai, the common Tagalog name, means “to give life”. A detailed review article also documented the chemical and biological importance of this plant (18).

In the present study, we investigated the normoglycemic and anti-hyperglycemic effect of *T. crispa* extract on streptozotocin-induced diabetic rats. This study also investigated the effect of different fractions of *T. crispa* on lipid profile of diabetic rats.

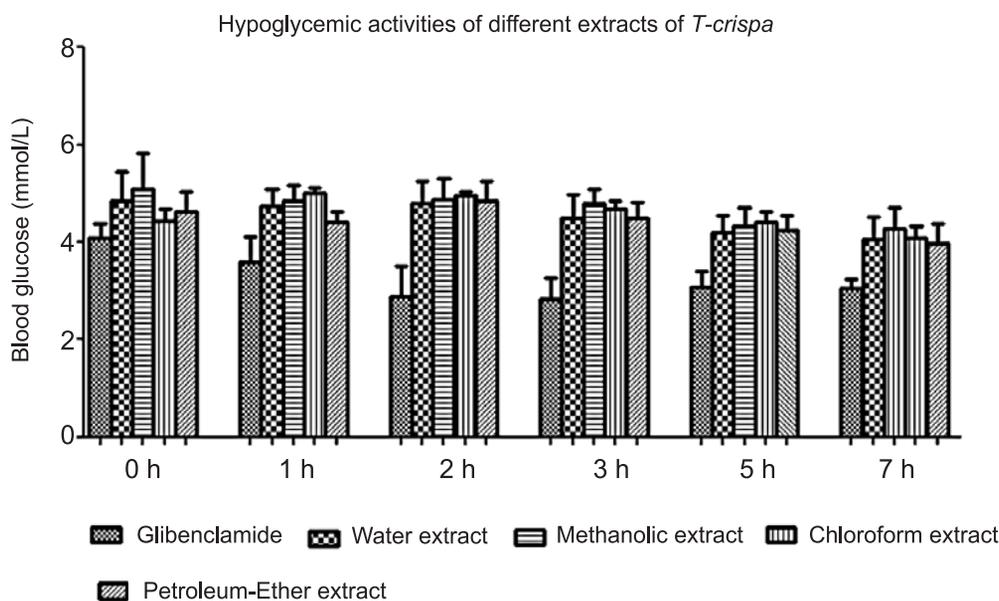


Figure 1. The effect of oral administration of, glibenclamide petroleum ether, chloroform, methanol and water extracts of *T. crispa* stems 1 g/kg, respectively, on glycemic effect on diabetic rats. Values are the means \pm S.E.M. of six animals

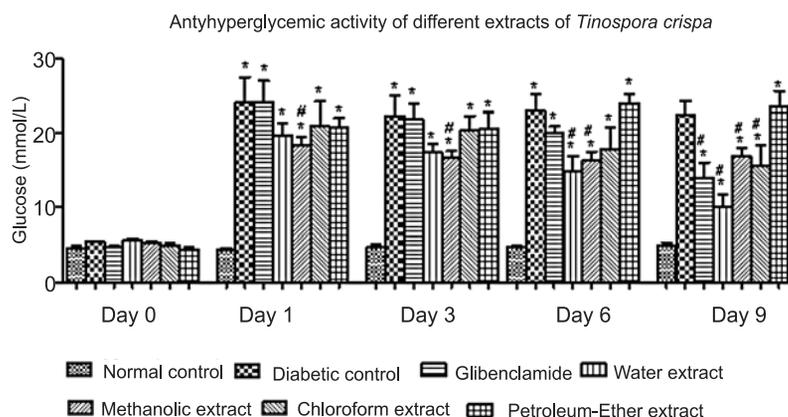


Figure 2. The effect of oral administration of: glibenclamide, petroleum ether, chloroform, methanol and water extracts of *T. crispa* stems 1 g/kg, respectively, on antyhyperglycemic effect on diabetic rats. Values are the means \pm S.E.M. of six animals. * $p < 0.05$ compared with normal control while # vs. diabetic control

MATERIALS AND METHODS

Plant materials

Overall weight of 10 kg of *Tinospora crispa* stems were purchased from Herbagus, Penang Island, Malaysia. One sample of the plant include stems, leaves and roots was send to Herbarium laboratory of USM and botanical identification of the plant was carried out by Dr. Rahad Zakaria. A voucher specimen of the plant (no. 11509) is deposited at the Herbarium of School of Biology Sciences, USM.

Preparation of plant extracts

The stems were washed and dried in an oven at 45°C. The dried materials were grounded into powder using Wiley Laboratory Mill apparatus to a coarse powder, and weighed and stored in dry airtight plastic containers until use. The dried stems powdered (1.95 kg) were extracted successively with petroleum ether followed by chloroform then with methanol and finally with water by maceration. The residue of the stems from the water bath extractions were further macerated in water at 50-60°C for three days but for water extract it was 80°C (10) and it was repeated three times. The mixtures were filtered with Whatman No. 1 filter paper three times to obtain the supernatant. Afterwards, the solvent was evaporated from each extract (using rotary evaporator) and the concentrated extracts were kept in a freezer at -70°C for 24 h. The concentrated frozen extract was then freeze dried under vacuum at -40°C for 24 h to give dried powdered extract. Dried extract were stored at -4°C until used.

Experimental animals

Male Sprague Dawley rats weighing (200-250 g) used in this study were obtained from the animal house Universiti Sains Malaysia. They were housed in clean cages and fed with food and water (temperature 25-30°C, 12 h light/dark cycle). Rats were randomly divided into six groups of six animals ($6 \times 6 = 36$) separately for the two categories of the experiments: hypoglycemic test on healthy rats (21) and anti-hyperglycemic test in diabetic rats.

Diabetes induction

In the anti-hyperglycemic set of the experiment, 36 male Sprague-Dawley rats were induced with diabetes by a single *i.p.* injection of streptozotocin (55 mg/kg) dissolved in cold normal saline, after an overnight fasting (10-12 h), as reported by (16). The blood glucose levels of the rats were determined 3 days later using blood from tail puncture and a glucometer. Rats with blood glucose level higher than 15 mmol/dL were considered diabetic and used for the experiment (16).

Experimental protocol

In the hypoglycemic test, rats were treated orally with the plant extracts (1.0 g/kg) suspended in Tween 80. Negative control group was treated with 10% Tween 80 in distilled water and positive control group with glibenclamide (10 mg/kg). Blood samples were collected from the tail vein at 0, 1, 2, 3, 5 and 7 h after treatment for blood glucose measurement and this treatment was continued for nine days and blood samples collected every three days

(14). In anti-hyperglycemic test, the diabetic rats were fasted overnight (12 h), rat tails were cleaned with 70% alcohol and a small cut was made on tail vein to obtain 10 μ L blood samples. Blood was taken to measure glucose level using ACCU-CHEK® Advantage III. The test groups of 6 diabetic rats were treated orally with extracts of *T. crispa* (1.0 g/kg), glibenclamide (10 mg/kg) and 10% Tween 80, (10 mL/kg), respectively. Blood samples were collected from the tail vein at 0, 1, 2, 3, 5 and 7 h after administration for blood glucose measurement and the treatment was continued for nine days and blood samples were collected every three days to the end of experiment. Whole blood collected directly from heart by using syringe and needle (No. 16) into heparinized tubes was centrifuged and 2 mL plasma separated from each blood tube was used for glucose and lipid profile tests.

Statistical analysis

Data were analyzed by using the SPSS and GraphPad Prism software and differences between treatment and controls were considered significant at $p < 0.05$. Lipid profile data were analyzed by using one way ANOVA and Boneferoni *post hoc* test.

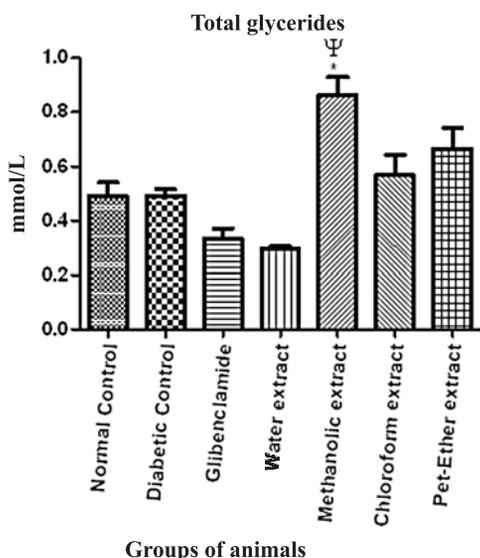


Figure 3. The effect of oral administration of petroleum ether, chloroform, methanol and water extracts of *T. crispa* stems 1 g/kg, respectively, on triglyceride level of diabetic rats. Values are the means \pm S.E.M. of six animals. * $p < 0.05$ compared with normal control, Ψ $p < 0.05$ vs. diabetic control

RESULTS

Figure 1 shows normoglycemic effects of different fractions of the plant. The extracts had no significant effect on blood glucose level during 7 h test comparing to normal control group, treated by normal saline and positive control group which was receiving glibenclamide 10 mg/kg. It also shows that 10 mg/kg glibenclamide is lowering glucose level significantly compared to normal control.

The effect of administration of extracts of *Tinospora crispa* on blood glucose level of diabetic rats is shown in Figure 2. From the day 6, water and methanol extracts significantly lowered blood glucose level. However, on day 9, water extract along with glibenclamide displayed strong blood glucose lowering effect ($p < 0.05$) as compared to diabetic control group.

Figures 3-6 show changes of triglyceride, LDL, HDL and cholesterol in blood samples of diabetic rats after 9 days treated by petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (1 g/kg) compared with diabetic control (normal saline) and glibenclamide. According to Figure 3 and 4, respectively, there is no significant effect on total triglyceride and LDL

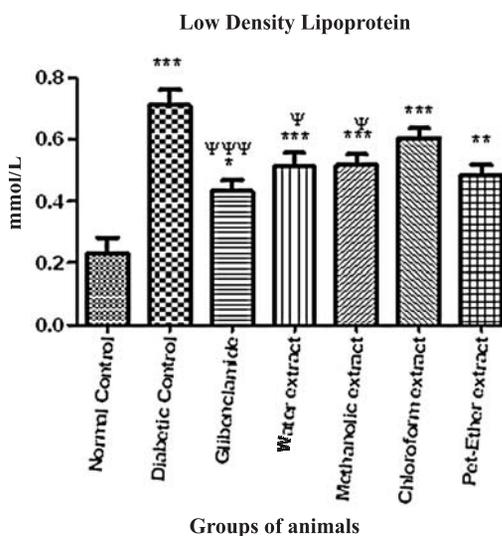


Figure 4. The effect of oral administration of petroleum ether, chloroform, methanol and water extracts of *T. crispa* stems 1 g/kg respectively on LDL level of diabetic rats. Values are the means \pm S.E.M. of six animals. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$ compared with normal control while Ψ $p < 0.05$, $\Psi\Psi\Psi$ $p < 0.0001$ vs. diabetic control

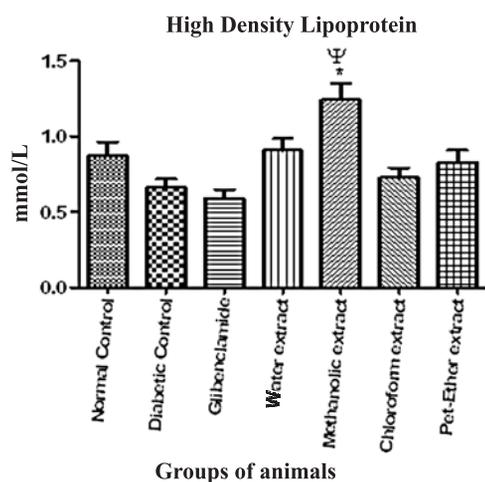


Figure 5. The effect of oral administration of petroleum ether, chloroform, methanol and water extracts of *T. crispera* stems 1 g/kg, respectively, on HDL level of diabetic rats. Values are the means \pm S.E.M. of six animals. * $p < 0.05$ compared with normal control while Ψ vs. diabetic control

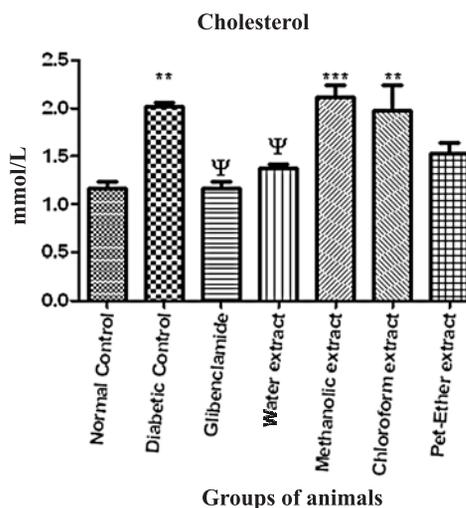


Figure 6. The effect of oral administration of petroleum ether, chloroform, methanol and water extracts of *T. crispera* stems 1 g/kg, respectively, on cholesterol level of diabetic rats. Values are the means \pm S.E.M. of six animals. ** $p < 0.05$, *** $p < 0.001$ compared with normal control; Ψ $p < 0.05$ vs. diabetic control

although in Figure 5 we observed some significant increase in HDL level of diabetic rats treated with methanol and chloroform extract. Also in Figure 6, we have the same significant increase of blood cholesterol level treated by methanol extract of *T. crispera*.

DISCUSSION

The present study was aimed to demonstrate the most active and potential extract of *T. crispera* plant (out of four extracts) as an oral anti-hyperglycemic agent effecting diabetic rats. This study has shown that when petroleum ether, chloroform, methanol and water extracts were given to normoglycemic rats they shown no hypoglycemic activity and there was no statistically significant and scientifically meaningful reduction in blood glucose level. Our data is in line with some other study (15) in which different fractions of plant has shown no hypoglycemic activity even after several hours of administration. These data are confirming the evidence that capsule of *T. crispera* when given to healthy rats make individual glucose level and insulin level in the plasma unaffected (1). In the contrary to extracts of this plant, the positive control drug has shown hypoglycemic effect which is in line with mode of action of the drug. Glibenclamide is the sulfonylurea and has an ability to sensitize the β cells

of Islets of Langerhans to secrete more insulin to metabolize the glucose inside the body. Thus, it is clearly indicated that four extracts of plant *T. crispera* has no ability to sensitize these β cells to secrete insulin.

In subsequent step, diabetes was induced by streptozotocin (STZ) which causes the necrosis of β cells of pancreas. The STZ is a glucose analogue, accumulates in pancreas and dissociates into glucose and methylnitrosourea. This methylnitrosourea alkylates and modifies biomolecules (destroy DNA) and destroy β cells thus causing diabetes (17). The glibenclamide being positive control as standard drug failed to bring the glucose level to normal or close to normal value. The logic behind this failure is destruction of β cells and lack of ability of β cells to secrete insulin. However, among all the plant extracts, water extract of *T. crispera*, at the dose of 1 g/kg, effected the glucose level less than glibenclamide. This shows that when β cells are destroyed, then the water extract can reduce the glucose level by a different mechanism which is still unclear. However, these data are in line with previous study on human in which insulin level in plasma was elevated after taking aqueous extract of *T. crispera* (13). The mechanism by which water extract of *T. crispera* reduces the blood glucose levels even when β cells are destroyed remained unclear. In these results, there was no significant effect on blood glucose lev-

els in diabetic rats treated with petroleum ether, chloroform and methanol extracts.

The dyslipidemic effect of four extracts of *T. crispa* showed variable data in respect to pharmacological response. The total glyceride levels were lower in chloroform and water extract of *T. crispa* but this decrease was not significant, although it was meaningful enough to be counted. The lethal cholesterol LDL level was not reduced by using all four fractions indicating that none of the extracts has dyslipidemic activity. In contrary to all good cholesterol, HDL level was improved by using all the extracts but the highest levels of HDL were observed with methanol, chloroform and water extracts, respectively, as compared to glibenclamide treated and diabetic rats. This indicates that this plant has potential of reducing cholesterol level by improving HDL levels. However, the most active fraction and different doses of that fraction need to be elucidated to get therapeutic responses.

Furthermore, this study extended the effects of all extracts of *T. crispa* on lipid profile of diabetic rats. The reason behind this idea was that every diabetic patient should be given statin to avoid secondary complications. Water extract has been found to be effect in modulating the lipid profile as compared to other fractions. Although methanol extract have been found to increase HDL levels in plasma, but at the same time has been blamed for increasing TG levels as compared to diabetic control. Lipid profile found to be well controlled by administering water extract irrespective of the fact that no statin as positive control is being used. The myth of the study was to compare best fraction of *T. crispa* which has not only antihyperglycemic control but also has ability to reduce cholesterol levels as compared to diabetic control and glibenclamide.

CONCLUSION

Present study confirmed that none of the fraction of *T. crispa* has hypoglycemic effect but water extract of *T. crispa* has antihyperglycemic effect along with the ability to reduce the lipid profile in diabetic rats.

Our results suggest that *Tinospora crispa* water extract can affect serum glucose levels in diabetic rats with type 2 diabetes mellitus and provide better lipid control in diabetic rats.

Acknowledgment

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