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Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Document heading doi: 10.1016/S2221-6189(13)60127-4

Effect of standardized extract of *Cosinium fenestratum* stem bark on liver and kidney function parameters in streptozotocin–induced diabetic rats

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ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form 21 April 2013
Accepted 20 May 2013
Available online 20 September 2013

Keywords:
Coscinium fenestratum
Diabetic rats
Hypoglycaemic
Liver and kidney functions
Standardized extract

ABSTRACT

Objective: To investigate the effects of standardized dichloromethane (DCM) extract of *Cosinium fenestratum* (*C. fenestratum*) stem bark on liver and kidney function parameters in streptozotocin (STZ)–induced diabetic rats. **Methods:** Standardization of the extract was performed through high performance liquid chromatography (HPLC) using berberine (BE) as marker compound. The standardized C. fenestratum stem bark extract (SCFE) was administered orally at a dose of 100 mg/kg to STZ–induced diabetic rats for 15 d. **Results:** The quantity of BE in the extract was 2.09% w/w. Blood glucose levels, blood urea nitrogen (BUN), alkaline phosphatase (ALP) and creatinine were significantly (P<0.05) elevated in STZ–induced diabetic rats as compared to normal control groups. Treatment of diabetic rats with the extract significantly (P<0.05) reduced, ALP, creatinine, and BUN levels as compared to the diabetic control group. The total white blood cells (WBC) count was reduced in diabetic rats. Treatment of diabetic rats with SCFE significantly (P<0.05) increased the total WBC count as compared to the values of diabetic control rats. **Conclusion:** The observations of the present study show that extract of *C. fenestratum* stem bark has hepato–renal protective effect in STZ–induced diabetic rats.

1. Introduction

Streptozotocin (STZ) induce production of oxygen radicals in the body that cause pancreatic injury responsible for the decrease in the insulin production leading to increase in blood sugar levels (hyperglycaemia). The hyperglycaemia in turn leads to increase in production of reactive oxygen species (ROS) which represent the main mediators of cellular injury. Increased in production of ROS impaired antioxidant defense systems and the lipid peroxidation induction which represent the main mechanism in many types of diseases, including the renal, cardiovascular diseases, cancer, the aging process and ROS mediated diabetic pathogenesis[1,2].

Cosinium fenestratum (*C. fenestratum*) Colebr. (Menispermaceae), is a woody climber found in India and Southeast Asia. The stem bark is widely used in traditional medicine. The infusion and tincture preparation of the stem bark is used in the traditional Ayurvedic system for the treatment of diabetes mellitus[3,4]. In the Siddah system of medicine, the powdered stem of the plant is used to treat diabetic patients[5]. Protoberberine alkaloids including berberine have been reported as the main constituents of the stem of *C. fenestratum*[6,7]. Tertiary alkaloids berlambine, dihydroberlambine and noroxyhydrastinine have also been isolated from the roots[8].

In our previous studies, the stem bark extract of *C. fenestratum* showed hypoglycaemic effect on streptozotocin (STZ)–induced diabetic rats[9]. Streptozotocin induced acute hyperglycaemia affects the functioning of the liver and kidneys due to the excess production of reactive oxygen species. Therefore, the aim of the present study

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was to evaluate the effect of standardized dichloromethane (DCM) extract of *C. fenestratum* stem bark on liver and kidney function parameters in streptozotocin (STZ)–induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Dichloromethane, methanol (HPLC grade) tragacanth powder and Tween 40 were obtained from Merck, (Darmstadt, Germany). Streptozotocin and tolbutamide were purchased from Sigma–Aldrich, USA. All others chemicals were of analytical or HPLC grade.

2.2. Plant material

C. fenestratum (20 kg) were obtained from Laboratory of Natural Products (NATPRO), Institute of Bioscience, University Putra Malaysia (UPM), Selangor, Malaysia. The plant was identified by Mr. Shamsul Khamis from NATPRO, UPM, Selangor, Malaysia. A voucher specimen is kept in the Institute for future reference.

2.3. Preparation and partial purification of extract

The plant material (stem) was air–dried at 25 ℃ for a week. The dried stem was cut into pieces and ground into powder form using a miller. Dried ground stem powder (1 kg) was extracted with DCM (4 L) for 48 h at room temperature.

The extract was filtered through Whatman filter paper and concentrated using rotary evaporator at 37 ℃ to yield a crude DCM extract (24.5 g). The DCM extract was partially purified using column chromatography technique with silica gel as stationary phase and methanol as mobile phase. Eluent was concentrated with rotary evaporator until dry at temperature 37 ℃ and stored at −20 ℃ until analysis.

2.4. HPLC analysis

Beberine (BE) was used as a standard marker compound. The separation and quantification of BE from the partially purified extract of *C. fenestratum* stem was performed by using Agilent 1200 HPLC System equipped with G1311A quaternary pump with G1322A degasser and G1315D diode array detector (DAD). The chromatographic separation was achieved with ODS column, Chromolith, RP–18e, 100 mm × 4.6 mm from Merck (Darmstadt, Germany). The mobile phase comprising of HPLC grade methanol and deionized water (90:10, v/v) with a flow rate of 0.5 mL/min and a column temperature of 25 ℃. The mobile phase was filtered under vacuum through 0.45 μm pores nylon filter membrane. UV detection was carried out at 254 nm. A volume of 20 μL of sample solution was injected each time.

Authentic BE standard solution and partially purified extract solution (1 mg/mL) were prepared in HPLC grade methanol. Standard solutions of BE in the range of 50–500 μg/mL were prepared and injected into HPLC system to establish a calibration curve. Peak area versus marker concentration was subjected to least square linear regression analysis and the slope, intercept and correlation coefficient for the calibration curve were determined.

The quantity of BE from the extract was expressed as %w/w. All measurements were performed in triplicates. The sample solutions and standard solutions were filtered using 0.45 μm syringe filter (Merck, Darmstadt).

2.5. Animals

Albino Wistar rats of both sexes weighting (180–200 g) were purchased from Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. They were housed in standard metal cages at (26 ± 2) ℃ and maintained under standard 12 h light/12 h dark cycle throughout the duration of the study.

All animals were given access to food and water *ad libitum*. They were deprived of food but not water before the commencement of the experiment. The drugs and test substances were administered orally. All the procedures involved animal testing in this study were reviewed and approved by Faculty Research Ethics Committee.

2.6. Toxicity studies

Rats of either sex (n=6), starved overnight were orally fed with SFCE at a dose of 5 000 mg/kg body weight for 15 d. The rats were observed for 4 h for behavioral, neurological and autonomic profiles. The animals were observed for lethality (death) after a period of 72 h.

2.7. Induction of hyperglycaemia in rats

The initial bodyweight and blood glucose level of experimental rats were measured and noted. The rats were then fasted overnight for at least 12 h prior to STZ induction[10].

The induction of diabetes was performed via a single intraperitoneal (i.p.) injection whereby each rat received a dosage of 65 mg/kg bodyweight STZ.

The blood glucose level and the bodyweight of the rats were assessed 72 h post–STZ injection to confirm hyperglycaemia and only rats with elevated blood glucose levels of above 11.0 mmol/L or 200 mg/dL were used for this study. All the rats had free access to water and food after the administration of STZ.

2.8. Study design, treatment and measurement of blood glucose level of experimental rats

In the experiment, 20 rats (15 diabetic rats, 5 normal rats) were divided into four groups of 5 rats each (n = 5).

Group I: Normal rats treated with 0.9% saline.

Group II: STZ-induced diabetic rats administered with 0.9% saline.

Group III: STZ-induced diabetic rats administered with tolbutamide (100 mg/kg).

Group IV: STZ-induced diabetic rats administered with SFCE (100 mg/kg).

Before the commencement of the treatments, blood samples for glucose determination were obtained from the tail tip of the 12 h fasted rats on day-0 (before STZ administration) and day-3 (72 h post STZ administration). The blood glucose levels of the rats were determined immediately by using a quantitative glucose meter, Accutrend® GCT and Accutrend® Glucose test strips coded 852 (Roche Diagnostics, Germany).

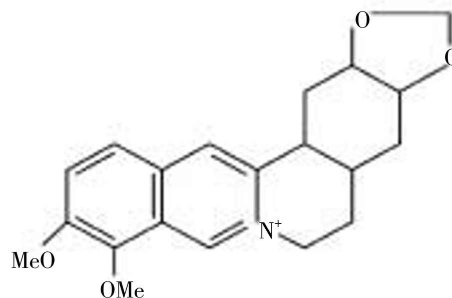
Bodyweights of all the experimental rats were also measured on day-0 (before STZ administration) and day-3 (72 h post STZ administration). Diabetic rats in negative control group (Group II) and positive control group (Group III) were administered with 0.9% saline and 100 mg/kg tolbutamide, respectively.

The diabetic rats of the remaining group (Group IV) were administered with 100 mg/kg of SCFE. Each drug was administered orally once a day for 15 d. Blood samples for glucose determination were obtained from the tail tip of the diabetic rats on day 1, 5, 10 and 15 of the treatment. Bodyweights of all the experimental rats were also measured on day 1, 5, 10 and 15 of the treatment.

2.9. Liver and kidney functions test

After the determination of glucose and body weight on the 15 d, the rats were anaesthetized with diethyl-ether vapour and blood was obtained by cardiac puncture. Blood was quickly transferred to blood collection tubes containing heparin and the tubes were placed in ice and sent to UCSI University Path Lab (Kuala Lumpur, Malaysia) for liver and kidney function analysis.

The following parameters were estimated total white blood cells (WBC) count, alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine. The liver function test was analysed by using Roche Cobas C311 chemistry analyzer (Roche, Switzerland) and the total WBC count was analysed by using Abbot Cell-DYN 3200 hematology analyzer (Abbott Laboratories, USA).



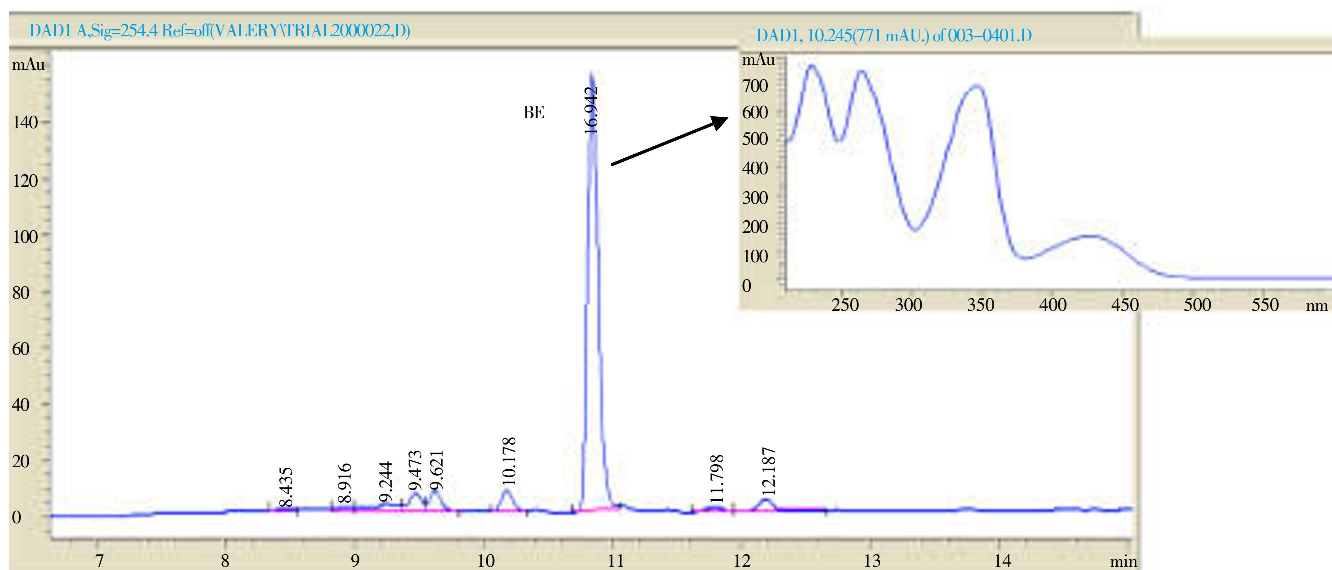


Figure 1. (A) Chemical structure of berberine; (B) HPLC/DAD profile of dichlomethane extract of *C. fenestratum* at 254 nm. Insert shows UV spectra of berberine component.

2.10. Statistical analysis

Statistical significance was assessed using one–way analysis of variance (ANOVA) and Dunnett test to compare the data. Probability (P) values of less than 0.05 were considered significant difference between means. All values are presented as means ± standard error the mean (SEM).

3. Results

3.1. HPLC Analysis

Figure 1 shows a typical HPLC chromatogram of the extract. The identification of BE was based on comparison of retention times and UV spectra of peak of BE standard with the corresponding peak in the sample chromatogram. Quantitation was performed on the basis of linear calibration plots of the UV absorption peak area at 254 nm against concentration. Linear correlation between the peak area and concentrations gave correlation coefficient of 0.999 which indicates a good linearity. The retention time (tr) for BE was 10.94 min. The quantity of BE from the extract was found to be 2.09% w/w.

3.2. Acute toxicity studies

Rats treated orally with SCFE (5 000 mg/kg body weight) showed no signs and symptoms of toxicity over the period of treatment (14 d). There was no abnormality in any of the organs indicating the *C. fenestratum* extract is not toxic. No significant difference was observed in the relative organ weights, body weight gain, food intake and water consumption between SFCE treatment groups and control group. None of the treated rats died during the period of observation after the administration of SCFE.

3.3. Effect of extract on bodyweight and blood glucose levels

Figures 2 and 3 show the effect of treatment of SCFE on on body weights and blood glucose levels of experimental rats. The bodyweights of rats showed significant decrease 72 h post–STZ induction compared to prior diabetes induction by STZ (day 0). The fasting blood glucose levels of the experimental rats were determined before induction of diabetes by STZ at day–0 and day–3 (72 h) post–STZ induction. It was observed that STZ administration at a dosage of 65 mg/kg showed significant increase in blood glucose levels 72 h post–STZ induction compared to levels prior to diabetes induction (day–0).

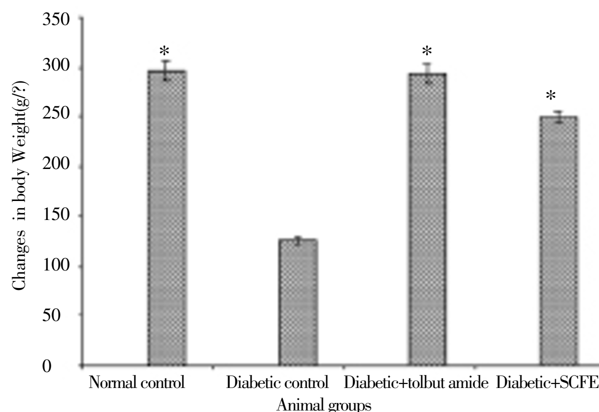


Figure 2. Effect of treatment of dichlomethane extract of *C. fenestratum* extract on (A) body weights of STZ–induced diabetic rats.

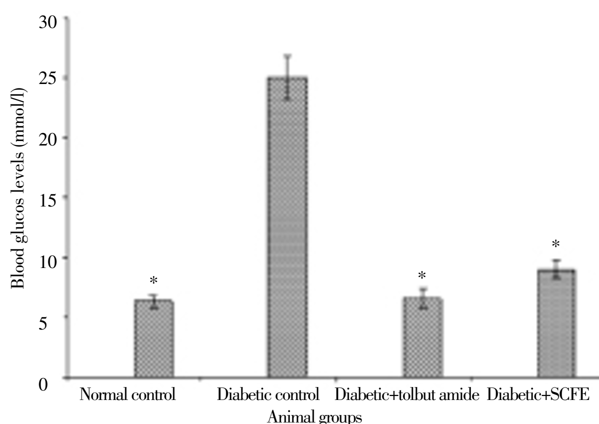


Figure 3. Effect of treatment of dichlomethane extract of *C. fenestratum* extract on blood glucose levels of STZ–induced diabetic rats.

Oral administration of tolbutamide (100 mg/kg) and SCFE (100 mg/kg) to the STZ–induced diabetic rats showed significant decrease in blood glucose levels and significant increase in bodyweights. Saline did not show significant reduction in blood glucose levels and reversal of bodyweights of diabetic rats during the 15 d treatment. The extract significantly (P<0.05) reduced blood glucose

Table 1.
Effect of 15 days treatment of *C. fenestratum* extract on biochemical parameters in normal and STZ induced diabetic.

Groups (n=5)	Total WBC ($\times 10^9$/L)	Alkaline phosphate (ALP) (IU/L)	Creatinine (μ mol/L)	Blood urea nitrogen (BUM) (mmol/L)
Normal control	9.9 ± 0.6	131.7 ± 2.4	55.0 ± 0.4	4.9 ± 0.3
Diabetic control	5.9 ± 0.5	938.7 ± 4.8	64.7 ± 1.2	12.7 ± 0.5
Diabetic + tolbutamide (100 mg/kg)	7.1 ± 0.4*	604.8 ± 5.4**	54.6 ± 0.9*	8.3 ± 0.5*
Diabetic + SCFE (100 mg/kg)	7.8 ± 0.6*	641.5 ± 6.1**	60.3 ± 0.6*	10.3 ± 0.4*

Normal rats were administered with 0.9% saline. Diabetic control rats were administered with 0.9% saline. Each value represents Mean + standard error of the mean (SEM) of five rats per group. **P<0.01, *P<0.05, in comparison with diabetic control values.

levels of STZ-induced diabetic rats (8.2 ± 0.7) mmol/L as compared to diabetic control rats (25.0 ± 1.8) mmol/L.

3.4. Effect of extract on biochemical parameters

The effect of SFCE on total WBC, ALP, creatinine and BUN are shown in Table 1. The total WBC was reduced in diabetic rats as compared to normal rats. Treatment of STZ-induced diabetic rats with SCFE (100 mg/kg b.wt.) significantly ($P<0.05$) increased the WBC values. Treatment of STZ-induced diabetic rats with tolbutamide (100 mg/kg b.wt.) also elevate the WBC values significantly ($P<0.05$).

Levels of ALP, creatinine and BUN were elevated in STZ-induced diabetic rats as compared with the values in normal rats. Animals treated with SCFE (100 mg/kg b.wt) showed a significant ($P<0.01$) reduction in the elevated levels of ALP, creatinine and BUN. Treatment of STZ-induced diabetic rats with tolbutamide (100 mg/kg b.wt.), significantly ($P<0.01$) reduced the elevated levels of ALP, creatinine and BUN.

4. Discussion

The objective of the HPLC part of this study was to standardized the DCM extract using BE as a marker before administer to the rats. BE was selected as authentic marker compound because it has been reported as the major bioactive chemical component of *C. fenestratum*[6,7]. The DCM extract was standardized with HPLC to achieve a BE concentration of ≥ 2.0 % w/w. BE is a natural isoquinoline alkaloid present in roots and stem bark of *Berberis* species and it has shown a variety of pharmacological activities[11].

The toxicity studies confirmed that the *C. fenestratum* extract is safe. Administration of SFCE showed no lethality or adverse reactions observed at the selected dose during the period of observation. All the rats treated with 65 mg/kg STZ displayed features associated with uncontrolled diabetes mellitus, such as elevation of blood glucose levels of above 11.1 mmol/L and slight loss in bodyweight 72 h post STZ administration[12]. We also observed symptoms such as; fatigue, slow movement, increased consumption of food (polyphagia), hyperglycemia, and excessive thirst (polydipsia).

The oral administration of saline did not reduce the blood glucose levels or increase in body weights of STZ-induced diabetic rats after 15 d treatment. Rather there was an elevation of blood glucose and a reduction of bodyweight. This shows that there were no hypoglycaemic effects or reversal in bodyweight of the diabetic rats exerted by the negative control (saline). The orally administration of tolbutamide (positive control) at 100 mg/kg to the diabetic rats significantly decrease their blood glucose levels and slightly increased their body weight after the 15 d of treatment. Tolbutamide is an oral anti-hyperglycemic agent of the first generation sulfonylurea. Its principal action is to increase insulin release from the pancreatic β-cells, reduce serum glucagon levels and closure of potassium channels in extra pancreatic tissues to give the combined plasma glucose lowering effect[13].

The oral administration the SCFE at 100 mg/kg to the diabetic rats significantly reduced blood glucose lowering levels and increase bodyweights of STZ-induced diabetic rats after the 15 d treatment. The reversal of blood glucose level and weight loss in diabetic rats treated with SCFE suggests that the extract has hypoglycaemic activity. It can be suggested that the extract has similar mechanisms to that of tolbutamide which promoted glucose uptake by enhancing the release of insulin from the pancreatic beta cells of the rats. Excessive hepatic glycogenolysis and gluconeogenesis is associated with decreased utilization of glucose by the tissues of the diabetic rats which caused its bodyweight to decrease and its reversal may have been the cause for the increase in bodyweights of the diabetic rats[14]. The blood glucose lowering effect of the SCFE may be ascribed to the alkaloid content including BE which was reported to have similar effect to that of metformin[15]. Laboratory studies indicate that BE may have at least two functions in relation to reducing blood sugar: inhibiting absorption of sugars from the intestine and enhancing production of insulin[16].

Alkaline phosphatase test is used to detect liver damage. High ALP values mean that the liver is damaged. Damaged liver cells release increased amount of ALP into the blood. In this study, high ALP values were observed in diabetic control rats as compared to normal rats which reveal liver dysfunction in negative control rats. These values were significantly reduced in diabetic rats treated with the SCFE. The same was observed in rats treated with standard drug tolbutamide. Other studies have also reported that high ALP values in diabetic rats can be reversed when treated with plant extracts and standard drugs that have antidiabetic activity[17,18]. The results suggest that the extract has the ability to repair liver damage caused by STZ in diabetic rats. This effect of the extract can be ascribed to the chemical constituents including BE which was reported to have hepatoprotective effects on carbon tetrachloride-induced acute hepatotoxicity in rats[19]. BE administration *in vivo* protected pancreatic islets and serum lipids in Nonobese diabetic (NOD) mice[20].

Creatinine a metabolite of creatine is generated from muscle metabolism. As the kidneys become impaired, the creatinine levels in the blood rises due to poor clearance by the kidney causing abnormally high levels of creatinine which is an indication of possible malfunction or failure of the kidneys. Diabetes is one of the most common causes of longstanding kidney diseases. This is proved by the elevation of creatinine levels in negative control diabetic rats compared to normal rats. The creatinine levels were

reduced significantly in the diabetic rats treated with the SCFE which shows that the treatment reduced the oxidative damage to the kidney as well as the diabetic conditions.

Blood urea nitrogen (BUN) measures the amount of urea nitrogen (waste product of protein metabolism) in the blood. The liver forms urea but it is the kidney's function to remove it from the blood stream. So the amount of urea nitrogen in the blood can be used as a test of renal function, although many other factors can affect the BUN values including hydration status and liver failure. When kidney functions are impaired, they cause an elevated BUN because the kidneys are less able to clear the urea from the bloodstream. This condition was observed in the negative control rats where BUN values were increased significantly compared to normal rats indicating kidney problems. These values were significantly reduced in diabetic rats treated with SCFE.

In conclusion, 15 d treatment of rats with dichloromethane extract of *C. fenestratum* stem bark showed no lethality and adverse effects. Standardisation of the extract by HPLC confirmed BE as the as the major bioactive constituent in the extract. Treatment with the standardized extract showed blood glucose lowering activity and ability to repair liver and kidney damage in STZ-induced diabetic rats. The elevation of liver and kidney function parameters in STZ-induced diabetic rats were improved by the treatment of the diabetic rats with the extract.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

This work was financially supported by research grant from UCSI University (Proj-in-FAS-006).

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