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Contributed Paper

Amelioration of Oxidative Stress and Pathological Alterations in Alloxan-induced Diabetes Mice by *Tithonia diversifolia* Leaves Extract

Lanchakon Chunudom [a,b], Montakarn Thongsom [a,b], Naymul Karim [b], Jitbanjong Tangpong*[b,c]

[a] Biology Program, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat, 80280, Thailand.

[b] Biomedical Sciences Program, School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, 80161, Thailand.

[c] Research Excellence Center for Innovation and Health Product (RECIHP), School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, 80161, Thailand.

*Author for correspondence; e-mail: rjitbanj@wu.ac.th

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Abstract

Aim was to evaluate the ameliorative effect of *Tithonia diversifolia* leaves extract (TDE) in alloxan-induced diabetic mice. *Tithonia diversifolia* is a sunflower family plant, and has been used as traditional alternative medicine for treating numerous diseases including diabetes. In our study, *in vitro* antioxidant and free radical scavenging activities of TDE was evaluated. In *in vivo* research, alloxan-induced diabetic mice were treated with different dose of TDE (100, 200, and 300 mg/kgBW) and Glibenclamide 60 mg/kgBW for 4 weeks. At the end of treatment, mice were sacrificed, collected blood and tissues (liver and pancreas) sample for evaluating the metabolic profile, oxidative stress marker, antioxidant enzymes activity, and pathological alterations. Results showed the TDE exerted potent *in vitro* antioxidant and free radicals scavenging activities. In diabetic mice, TDE treatment significantly reduced blood glucose and increased the serum insulin levels. In addition, the treatment also modulated the hepatic oxidative stress related parameters e.g. reduced malondialdehyde level, and increased the catalase and glutathione peroxidase levels. Histopathological analysis revealed that TDE treatment significantly ameliorated the hepatic and pancreatic degradation in diabetic mice. Taken together, TDE could be an alternative supplement for the management of the diabetes and its complications. Nevertheless, further researches are required to find out the underlying mechanism.

Keywords: alloxan, oxidative stress, diabetes, pathological alterations, *Tithonia diversifolia*

1. Introduction

Diabetes mellitus (DM), a noncommunicable complex metabolic disease, are associated with sustained hyperglycemia because of insulin resistance and/or absence of insulin production [1]. Currently, it is not only a major health issue but also a clinical challenge for the world. According to WHO studies, by the year of 2025, the total

number of DM patients would reach up to 300 million. In addition, it would be the 7th leading cause of death worldwide by the year of 2030 [2]. Until now, the number of pharmaceutical agents are used for treating DM, but they may have unwanted side-effects. For example, treatment of metformin causes adverse effect such as nausea, vomiting, diarrhea, and rare hepatoxicity [3,4]. Thus, there is an increase interested in herbal remedies because of their minimal side-effects and maximal preventive outcome. Traditional plants with higher antioxidant compounds have an important role in treating diseases involve with oxidative stress e.g. DM. The potential role of antioxidant constituents of the medicinal plants for maintenance of health is a raising interest among patients and researchers.

Tithonia diversifolia (Hemsl) A. Gray (TD) is a member of the sunflower family, Asteraceae. It is well known as Mexican sunflower and this shrub is available in America, Asia, as well as in African countries. Nigerian people use TD as traditional alternative medicine for the treatment of sore throat, malaria, diabetes, menstrual pain, inflammation, and liver disease etc. after decoction of its different parts [5-7]. In addition, current research showed that TD and its derivative compound exhibited protective effect against oxidative stress, inflammation, obesity, neurodegeneration, and cancer [8-12]. Interestingly in the southern Thailand, decocted-TD leave was used for the treatment of hyperglycemia in diabetes patients, unfortunately it has lack support of laboratory research data. This is why current research first-time aimed to evaluate the antidiabetic effect of TD extract on alloxan-induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Folin-Ciocalteu, ABTS, Alloxan monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Honeywell Fluka Chemicals (Germany), and Merck & Co. (Germany) respectively, whereas, insulin ELISA kit was purchased from Millipore Corporation (Billerica, MA, USA). All analytical grade chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Merck & Co. (Germany) and Calbiochem and Co. (Germany) unless otherwise mention.

2.2 TD Collection and Extract Preparation

Tithonia diversifolia (TD) plant leaves were collected from Yala Province, Thailand. The herbarium number of TD is BKF No.186838 and was confirmed by the Office of Herbarium, Department of National Parks, Wildlife and Plant Conservation of Thailand. TD leaves were washed, dried, blending to get TD powder. TD was submerged into boiling distilled water. Then, the solution was filtered, centrifuged, frozen at -20°C and lyophilized, respectively.

Later, TD aqueous extract were added with n-butanol for 12h with constant shaking and then centrifuged at 3000 rpm for 30 min. Collected sediments was dissolved in a series of binary solution of n-butanol:ethanol, methanol:ethanol and methanol:water (50:50 v/v, 12h with constant shaking), respectively. Each time, collected sediment was centrifuged at 3000 rpm for 30 min. The final aqueous extract (TDE) was obtained by dissolving extract in distilled water for 12h with constant shaking and centrifuging at 3000 rpm for 30 min. Then, TDE was collected, frozen at -20°C and stored at -20°C. The TDE test sample was enough prepared for this experiment.

2.3 Determination of Total Phenolic Content and ABTS Scavenging Activity of TDE

Total phenolic content of TDE was evaluated by Folin-Ciocalteu's method while gallic acid was used as standard. Concisely, 20 µl of TDE was added with 1.55 ml double deionized water and 100 µl Folin-Ciocalteu reagent. The solution was mixed using vortex mixture and incubated for 5 min at room temperature. Following incubation, 300 µl of Na_2CO_3 (2 % W/V) solution was added with the mixture and allowed to stand at room temperature for 2h. After incubation,

absorbance was measured at 765 nm and the value was expressed as gallic acid equivalents of TDE (GAE mg/g TDE).

The total antioxidant activity of TDE was evaluated by ABTS radical cation decolorization assay. Shortly, ABTS working solution was prepared by mixing of 5 ml of 7 mM ABTS and 80 µl of 140 mM potassium persulphate ($K_2S_2O_8$) and kept it for 12-16h at room temperature in dark condition. Then, 1 ml of ABTS working solution was added to glass test tube containing 50 µl of TDE and the solution was mixed using vortex mixture. The absorbance was measured at 734 nm after 2 min. The percentage of radical scavenging activity of TDE was calculated by comparing the absorbance value with control. The value was expressed as µM of Trolox equivalent per mg of TDE (µM TEAC/mg TDE).

2.4 Determination of Hydroxyl Radical and Superoxide Anion Scavenging Assay of TDE

Hydroxyl radical scavenging assay was performed as according to the Fenton-type reaction. The reaction mixture contained 1 ml of 0.1 mM methyl violet, 0.5 ml of 5 mM $FeSO_4$, 0.5 ml of 1% H_2O_2, and 2 ml Tris buffer (pH 4.0). Then, 0.5 ml of TDE was added with total reaction volume 10 ml. The absorbance of the reaction mixture was measured at 565 nm. The absorbance of TDE and control (without TDE) solutions were set as As and Ao, whereas the absorbance without $FeSO_4$ and H_2O_2 was set as A. The hydroxyl radical scavenging activity (D) of TDE was calculated according to the following equation:

$$D = \frac{[As - Ao]}{[A - Ao]} X\ 100\%$$

For superoxide anion scavenging assay, 100 ml of 3 mM pyrogallol and 3 ml Tris buffer (pH 8.2) were mixed with 0.5 ml of TDE. The auto-oxidation rate of pyrogallol was measured by determining the changes of the absorbance at 325 nm within 4 min using an UV–Vis spectrophotometer. The absorbance with TDE and control (without TDE) were set as A2 and A1. The superoxide anion scavenging activity (D) of TDE was calculated by comparing ΔA_1/min (without TDE) and ΔA_2/min (with TDE) against the pyrogallol auto-oxidation using the following formula:

$$D = \frac{[\Delta A1 - \Delta A2]}{[\Delta A1]} X\ 100\%$$

2.5 Experimental Animals and Diabetes Induction

Experimental ICR adult male mice with body weights range from 25 to 30 g were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. Before one week of experiment, mice were acclimated and allowed to access normal diet and water ad libitum. The animals were maintained 12h light/dark cycles in a controlled environment conditions, temperature (23 ± 2 °C) and humidity (55 ± 10 %). The experimental procedures were approved by the Animal Care and Use Committee of Walailak University, Thailand (No.005/2013).

The mice were injected with alloxan monohydrate at a dose of 120 mg/KgBW (dissolved in sterile normal saline). After 5th day of injection, the mice presenting blood glucose level above 200 mg/dl and decreased the body weight were included to the experiment.

2.6 Experimental Design

A total of 30 diabetic surviving and 6 normal mice were used for this experiment, whereas the diabetic surviving mice were randomly divided into five groups. Normal and diabetic control mice (1st and 2nd groups) received the distilled water instead of TDE. Treatment groups (3-5th groups) received the TDE at a dose of 100, 200, 300 mg/KgBW, respectively. In addition, the last group (6th group) received the standard drug glibenclamide at a dose of 60 mg/KgBW. Blood sample was collected from tail vein of all groups. The experimental group of mice (N = 6) are given below:

Group 1: Normal Control
Group 2: Diabetic Control (DM)
Group 3: DM+TDE 100 mg/KgBW
Group 4: DM+TDE 200 mg/KgBW
Group 5: DM+TDE 300 mg/KgBW
Group 6: DM+Glib 60 mg/KgBW

After 4 weeks of treatment, mice were fasted overnight and anesthetized using Nembutal sodium solution (65 mg/kg BW). The blood sample was obtained via left ventricle puncture into the heparin tubes. Serum sample was collected for analyzing of blood glucose and insulin levels, while liver and pancreas tissues were collected for histopathological analysis. Other lob of liver tissue was homogenized in cold phosphate saline (PBS, pH 7.4, containing protease inhibitors) prior to centrifugation at 13,500xg for 15 min at 4°C, and stored in -20°C until analysis.

2.7 Determination of Oxidative Stress Related Parameters in Liver Tissue

Before the evaluation of liver tissue oxidative stress marker, the liver were removed after sacrificing. Then, immediately perfused blood out of organ and washed by an ice-cold phosphate buffer saline.

Malondialdehyde (MDA) was measure as lipid peroxidation marker. Briefly, 200 µl samples of liver homogenate were mixed with trichloroacetic acid (1:1) and centrifuged at 3,000xg for 15 min. Then, 15 % of thiobarbituric acid was added to the supernatant, heated at 100°C in boiling water bath for 30 min, and centrifuged. The absorbance of supernatants was measured using spectrophotometer at 595 nm. The lipid peroxidation of samples was compared with a standard curve of MDA. The results were expressed as nM/mg protein.

Catalase (CAT) and Glutathione peroxidase (GPx) activity was measured as antioxidant marker. CAT activity was determined using the previously described procedure of Sinha (1972) with slight modification [13]. The assay mixture containing 4 ml of H_2O_2 solution and 5 ml of phosphate buffer, pH 7.0 in a 10 ml flat bottom flask. Immediately, 1 ml of diluted homogenate was mixed with reaction mixture by a gently swirling motion at room temperature. After that, 1 ml portion of the reaction mixture was withdrawn and added into 2 ml dichromate/acetic acid reagent and the absorbance was measured using spectrophotometer at 570 nm. CAT activity was expressed as U/mg protein. For GPx activity, the reaction mixture was prepared containing 100 µl of buffer, 20 µl of GSH, 100 µl of NADPH, 10 µl of homogenate, 10 µl of NaN_3, and 660 µl of distilled water. This mixture was warm at 37°C for 10 min, and then 10 µl of H_2O_2 was added. The absorbance was measured at 340 nm. The GPx activity was measured from the change in absorbance within 1 min, and the molar extinction coefficient for NADPH (6.22 mM cm^{-1}) at 340 nm was used for calculating GPx activity. The activity was expressed as U/g protein.

2.8 Histopathology Evaluations of Liver and Pancreas Tissues

The whole liver and pancreas from each animal were removed after sacrificing and immediately washed by an ice-cold phosphate buffer saline. A portion of liver and pancreas were selected at the same area and then fixed in 10% neutral formalin fixative solution for histological studies. After fixation, tissues were embedded in paraffin, solid sections were cut at 5 µm and the sections were stained with hematoxylin and eosin solution and analyzed by a Leica scanning confocal fluorescence microscope (Germany). Photos were captured at 10X magnifications for liver and pancreas sections. Histological evaluations were performed visually.

2.9 Statistical Analysis

All data are presented as mean ± SEM for sixth mice in each group of three independent determinations. Comparison between groups were done using one-way analysis of variance (ANOVA) followed by the Student-*t*-test. The Newman-Keuls multiple comparisons test was utilized to analyze the statistical significance

of data from experimental and control groups, while *P* values < 0.05 was considered as statistical significance. All statistical analyses were performed using SPSS (IBM SPSS for Windows, V. 17.0, New York, USA).

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content and Total Antioxidant Activity of TDE

The amount of total phenolic content and total antioxidant activity of TDE were determined. The results showed that the total phenolic content and total antioxidant of TDE were 46.01 ± 1.73 GAE mg/g TDE and 82.41 ± 2.084 μM TEAC/mg TDE, respectively (Table 1). In addition, it was observed that total antioxidant activity was positively correlated with the total phenolic content of TDE ($R^2 = 0.84$).

3.2 Hydroxyl Radical and Superoxide Anion Scavenging Activities of TDE

The activity of TDE was efficient in scavenging hydroxyl radical and superoxide anion. The hydroxyl radical and superoxide anion scavenging activities of TDE were showed as percentage of inhibition and the values were 99.69 ± 4.50% and 45 ± 3.93%, respectively (Table 2). These results suggested that TDE has scavenging capacity and can scavenge the hydroxyl radical and superoxide anion.

Antioxidants play a major role in protection against molecular oxidative damage [14,15]. The present study showed that TDE contained the higher level of total phenolic content which is correlated with the total antioxidant capacity. The total phenolic and flavonoid contents of TD leaves are different depending on type of extract. Aqueous extract was resulted richer in phenolic and flavonoid compounds compared to methanol and dichloromethane extracts. Consistent with their different polyphenol contents, the aqueous extract has proven the most effective scavenger than methanolic and dichloromethane extracts [16]. Similary results of the aqueous extracts of *Momordica Foetida* Schumach. et Thonn demonstrated the highest potential antioxidant activity compared to methanolic and dichloromethane extracts inhibit *in vitro* plasma lipid peroxidation [17].

Table 1. *In vitro* total phenolic content and antioxidant capacity of TDE.

Extract	Total phenolic content (mg of GAE/mg dry weight)	Total anti-oxidant capacity (μM TEAC/mg dry weight)
TDE	46.01 ± 1.73	82.41 ± 2.084

Results are expressed as mean ± SEM of three independent determination. TDE, Aqueous fraction of *Tithonia diversifolia* leaf extract; GAE, Gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity.

Table 2. *In vitro* radicals scavenging effects of TDE.

Extract	Hydroxyl radical scavenging (% of inhibition)	Superoxide anion scavenging (% of inhibition)
TDE	99.69 ± 4.50	45.00 ± 3.93

Results are expressed as mean ± SEM of three independent determinations. TDE, Aqueous fraction of *Tithonia diversifolia* leaf extract.

In addition, TDE exhibited the hydroxyl radical and superoxide anion scavenging activity. Previous studies found that the leave aqueous, methanolic and petroleum ether extracts of TD contained saponin, alkaloids, saponin glycoside, tannin and balsam. The most abundant among the phytochemical constituents in the leaves of TDE was saponins which significantly increased insulin sensitivity. Also, these phytochemicals had pharmacological effects including radical scavenging activities [18].

3.3 Effects of TDE on Body Weight Change and Metabolic Parameters of Diabetic Mice

Body weight change of control and diabetic mice were measured at 0 and 4 weeks. In normal mice, the body weight is consistency increased from 0 to 4 weeks when compared with 0 week. In case of diabetic mice, the body weight change is not significant throughout the study when compared with control group. However, the body weights of TDE-treated groups were comparatively improved when compared with 0 week (Table 3).

Fasting blood glucose and serum insulin levels of experimental mice were measured as metabolic parameters. In alloxan-induced diabetic control group, there was a significantly *(P<0.05)* increment of fasting blood glucose and decrement of serum insulin levels found when compared with the normal control group. Four weeks treatment of diabetes mice with TDE, dose-dependently ameliorated the higher fasting blood glucose and lower serum insulin levels compared with diabetic control group. In contrast, diabetic mice treated with 300 mg/KgBW of TDE were exerted the similar level of fasting blood glucose to the glibenclamide 60 mg/KgBW treated diabetic mice. In case of serum insulin level, TDE doses 200 and 300 mg/KgBW showed the similar level of serum insulin to the glibenclamide 60 mg/KgBW treated group. However, positive control glibenclamide demonstrated the lowest blood glucose and highest serum insulin levels than TDE-treated groups (Table 4). Diabetes is a metabolic abnormality characterized by elevated blood glucose because of defect in insulin secretion and/or action. The worldwide prevalence of diabetes is increasing rapidly [19]. Till date, diabetic patients are depending on pharmaceutical agent for treating diabetes. Unfortunately, these agents are engaged with several adverse effect which could be solvable by herbal remedies. This is because herbal remedies have minimal side-effects and maximal preventive outcome [20]. Hyperglycemia is known to produce reactive oxygen species (ROS) which play major role in diabetic complications from glucose auto-oxidation and protein glycolylation [21,1]. Earlier study exposed that oral administration of

Table 3. Effects of TDE on body weight change of alloxan-induced diabetic mice.

Treatment	Body weight at 0 week	Body weight at 4 week
Normal Control	32.06 ± 1.23	41.12 ± 2.63[a]
Diabetic Control (DM)	29.80 ± 2.68	33.28 ± 3.17
DM+TDE 100 mg/KgBW	30.20 ± 2.80	33.19 ± 1.95[b]
DM+TDE 200 mg/KgBW	33.68 ± 0.98	37.13 ± 0.78[b]
DM+TDE 300 mg/KgBW	31.23 ± 2.58	37.31 ± 2.06[b]
DM+Glib 60 mg/KgBW	32.58 ± 1.97	35.41 ± 2.14

Results are presented as mean ± SEM of three independent determinations (N = 6). TDE, Aqueous fraction of *Tithonia diversifolia* leaf extract; Glib, Glibenclamide. [a]*P<0.05* compared to 0 week and [b]*P<0.05* compared within the same diabetic group.

Table 4. Effects of TDE on blood glucose and serum insulin levels of alloxan-induced diabetic mice.

Treatment	Blood glucose (mg/dl)	Serum insulin
Normal Control	110.91 ± 7.96	4.48 ± 0.24
Diabetic Control (DM)	330.52 ± 9.17[a]	4.53 ± 0.16
DM+TDE 100 mg/KgBW	297. 23 ± 5.22[a,b]	4.67 ± 0.18
DM+TDE 200 mg/KgBW	278.37 ± 6.19[a,b]	4.79 ± 0.19
DM+TDE 300 mg/KgBW	172. 25 ± 3.16[a,b]	4.90 ± 0.19 [a]
DM+Glib 60 mg/KgBW	167.32 ± 3.05[a,b]	4.97 ± 0.17[a,b]

Results are presented as mean ± SEM of three independent determinations (N = 6). TDE, Aqueous fraction of *Tithonia diversifolia* leaf extract; Glib, Glibenclamide. [a]*P<0.05* compared to normal mice and [b]*P<0.05* compared to diabetic control mice.

ethanol extract of TD for three weeks reduced the blood glucose and increased serum insulin levels in diabetic mice [22]. Recent study reported that ethanolic fraction of TD increased insulin sensitivity by reducing hyperglycemia in diabetic rats [23]. But no study focused about the antidiabetic effect of aqueous fraction of *Tithonia diversifolia* in alloxan induced type 1 diabetic mice. Alloxan is the most commonly employed agent for the induction of diabetes in experimental animal model. It leads to a reduction in plasma insulin concentration leading to a stable hyperglycemia state [24]. Increment of ROS in diabetic condition could be due to the high blood glucose induced auto-oxidation [25].

3.4 Effects of TDE on Hepatic Oxidative Stress-related Parameters of Diabetic Mice

The results show in Table 5. The hepatic MDA level was measured as oxidative stress marker. In alloxan-induced diabetes group, the hepatic MDA was significantly ($P < 0.05$) increased when compared to normal control groups. Treatment with TDE (100, 200 and 300 mg/KgBW) and glibenclamide (60 mg/KgBW) significantly ($P < 0.05$) ameliorated the higher MDA levels when compared to the diabetic control group.

Hepatic CAT and GPx enzyme activities were measured as antioxidant parameters. These parameters were significantly ($P < 0.05$) reduced in alloxan-induced diabetic mice when compared with normal control group. However, the diabetic group which was treated with TDE (100, 200 and 300 mg/KgBW) and glibenclamide (60 mg/KgBW) significantly ($P < 0.05$) increased the hepatic CAT and GPx enzyme activities when compared to diabetic control group (Table 5).

3.5 Effect of TDE on Hepatic and Pancreatic Histology of Diabetic Mice

The effect of TDE on hepatic histology are shown in Figure 1. In the present study, the liver structure was normal in the normal control group, while diabetic control group showed the marked hepatocellular necrosis, microcellular fatty change (FA), extensive vacuolization with disappearance of nuclei (NC), as well as disorder in overall hepatic structure. The administration of different dose of TDE improved pathological changes. Treatments of TDE 200 and 300 mg/KgBW showed the better improvement (e.g. less vacuolization with increased nuclei) compared to TDE 100 mg/KgBW group. However, treatment of standard drug glibenclamide 60 mg/KgBW showed similar hepatocellular architecture to the normal control group (Figure 1).

Induction of alloxan to normal mice causes cellular necrotic and beta cell destruction of pancreas. According to Figure 2, normal control group had no architectural change in histology. In a diabetic

Table 5: Effects of TDE on hepatic oxidative stress-related parameters of alloxan-induced diabetic mice.

Treatment	MDA level (nM/mg protein)	CAT activity (U/mg protein)	GPx activity (U/mg protein)
Normal Control	4.42 ± 0.58	0.42 ± 0.02	0.19 ± 0.14
Diabetic Control (DM)	6.57 ± 0.73[a]	0.38 ± 0.01[a]	0.03 ± 0.01[a]
DM+TDE 100 mg/KgBW	4.31 ± 0.74[b]	0.48 ± 0.01[b]	0.06 ± 0.01[b]
DM+TDE 200 mg/KgBW	4.67 ± 0.71[b]	0.52 ± 0.02[b]	0.04 ± 0.01[b]
DM+TDE 300 mg/KgBW	2.91 ± 0.24[b]	0.58 ± 0.01[b]	0.05 ± 0.02[b]
DM+Glib 60 mg/KgBW	1.77 ± 0.08[b]	0.52 ± 0.01[b]	0.09 ± 0.05[b]

Data are represented as mean ± SEM of three independent determinations (N = 6). [a]*P<0.05* compared to normal mice and [b]*P<0.05* compared to diabetic control mice. TDE, Aqueous fraction of *Tithonia diversifolia* leaf extract; MDA, malondialdehyde; GPx, Glutathione peroxidase; CAT, Catalase.

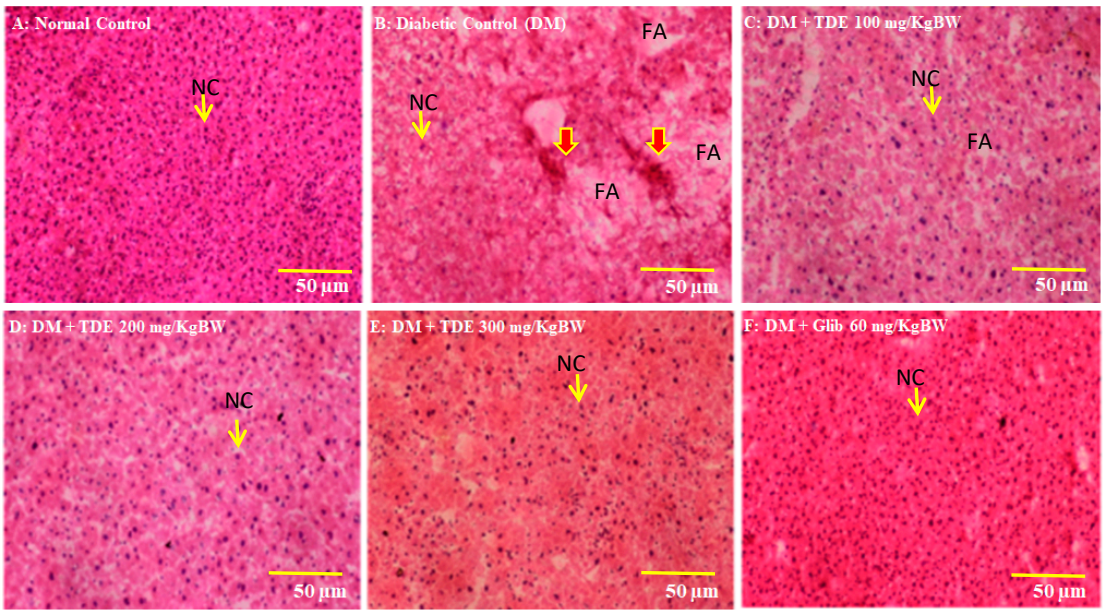

Figure 1 Histopathological evaluation of liver tissues of alloxan-induced diabetic mice. (A), Normal Control show normal liver structure; (B), Diabetic Control (DM) showed disorder in overall hepatic structure, marked hepatocellular necrosis (red arrow), microcellular fatty (FA), extensive vacuolization and disappearance of nuclei (NC); (C), DM + TDE 100 mg/KgBW; (D), DM + TDE 200 mg/KgBW; (E), DM + TDE 300 mg/KgBW; and (F), DM + Glib 60 mg/KgBW. All microscopic magnification, 10X.

control group, the Islet of Langerhans showed the higher degree of diffused necrotic changes compared to normal control group. Treatment of TDE (Dose 100 mg/KgBW) showed the mild degree of necrosis in the islet of langerhans when compared with diabetic control group. However, diabetic mice were treated treated with TDE (Dose 200 and 300 mg/KgBW) and glibenclamide 60 mg/KgBW showed no diffused necrotic and no architectural changes in pancreas (Figure 2).

Liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions. The biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes [26]. Lipid peroxidation refers to the oxidative degradation of lipid that impairs cell membrane functions resulting the cell damage and leading to several pathological alterations and cytotoxicity [27]. We also observed the increased hepatic lipid peroxidation in the hyperglycemic mice which was ameliorated by TDE treatment.

CAT is an enzyme antioxidant, which decomposes hydroxyl radicals. It is widely distributed in all animal tissues and the highest activity in the red blood cells and liver [28]. Reduction of CAT enzyme activity results in various deleterious effects due to accumulation of superoxide and hydroxyl radicals. In the present study, a reduced activity of CAT has been observed in diabetic control group and diabetic mice treated with TDE significantly increased the CAT level. The uncontrolled production of H_2O_2 was occurred

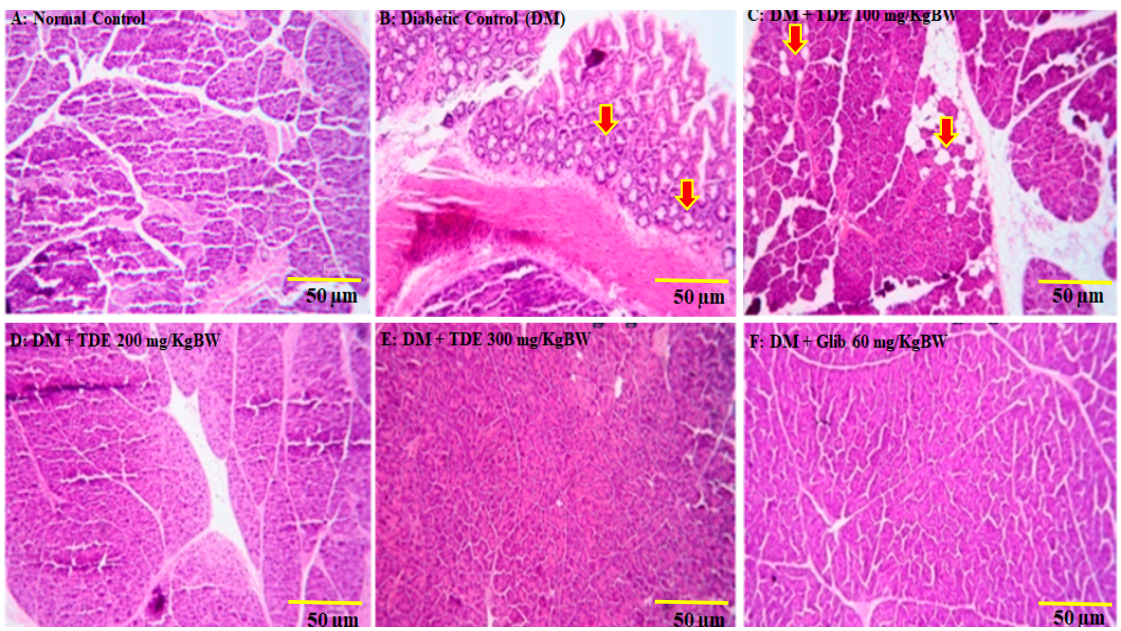

Figure 2 Histopathological evaluation of pancreas tissues of alloxan-induced diabetic mice. (A), Normal Control group had no architectural change in histology; (B), Diabetic Control (DM) group showed alloxan causes cellular necrotic and beta cell destruction of pancreas (red arrow); (C), DM + TDE 100 mg/KgBW; (D), DM + TDE 200 mg/KgBW; (E), DM + TDE 300 mg/KgBW; and (F), DM + Glib 60 mg/KgBW. The group which treated with TDE (Dose 200 and 300 mg/KgBW) and glibenclamide 60 mg/KgBW showed no diffused necrotic and no architectural changes in pancreas. All microscopic magnification, 10X.

in diabetic condition because of the glucose auto-oxidation, protein glycation, and lipid oxidation, which lead to a marked decline in the CAT activity [25]. GPx, a selenium containing tetrameric glycoprotein, detoxify the H_2O_2 from into water and molecular oxygen through the oxidation of reduced glutathione [29]. GPx has been shown to be an important adaptive response to condition of increased peroxidative stress. During diabetic conditions, the activity of GPx is decreased as a result of radical induced inactivation and glycation of the enzyme [30]. The low activity of GPx could be directly explained by the low content of glutathione found in a diabetic state since glutathione is a substrate and cofactor of GPx [31]. However, in our research, TDE treatment significantly increased the GPx activity compared to diabetic control group.

We examined that, alloxan-induced diabetic mice showed histological alterations in liver tissue (hepatocellular necrosis, microcellular fatty change, extensive vacuolization with disappearance of nuclei, and hepatic architectural damage) and in pancreas tissue (decrease in number and size of pancreatic islet and degranulation of β-cells). Four weeks TDE treatment to diabetic mice significantly improved the histological architecture of liver and pancreas compared to diabetic control group. The antidiabetic activity of TDE may be possible through the insulin mimetic action or other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis pathway in liver and muscle. The present study suggested that enrich of phenolic contents in TDE play a functioal role of antioxidant amilioate histopathological change of pancease and increased blood insulin level led to stimulate glucose uptake and reduced blood glucose level. Moreover, TDE showed improvement of liver tissue which confrimed previously studies indicated that polyphenol rich extracts exhibited the antidiabetic effect by inhibiting the histopathological alterations in diabetic liver, pancreas and kidney [32-35].

4. Conclusions

The present study demonstrated that TDE is the potential source of antioxidant and exerted free radical scavenging activity. In addition, TDE have the ability to reduce lipid peroxidation (MDA) and increase in the activities of CAT and GPx enzymes. Further study revealed that TDE modulated the diabetes induced histopathological alterations in liver and pancreas tissues. Thus, TDE could be an alternative supplement for the management of the diabetes mellitus. However, further studies are required to evaluate the underlying mechanism.

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References

[1] Karim N., Jeenduang N. and Tangpong, J., *Pol. J. Food Nutr. Sci.*, 2018a; **68(2)**: 163-169.

[2] Mathers C.D. and Loncar D., *PLoS Med.*, 2006; **3(11)**: e442.

[3] McCreight L.J., Bailey C.J. and Pearson E.R., *Diabetologia*, 2016; **59(3)**: 426-435.

[4] Miralles-Linares F., Puerta-Fernandez S., Bernal-Lopez M.R., Tinahones F.J., Andrade R.J. and Gomez-Huelgas R., *Diabetes Care*, 2012; **35(3)**: e21.

[5] Afolayan F.I.D., Adegbolagun O.M., Irungu B., Kangethe L., Orwa J. and Anumudu C.I., *J. Ethnopharmacol.*, 2016; **191**: 188-194.

[6] Lin H.R., *Bioorg. Med. Chem. Lett*, 2012; **22(8)**: 2954-2958.

[7] Zhao G., Li X., Chen W., Xi Z. and Sun L., *Fitoterapia*, 2012; **83(8)**: 1590-1597.

[8] Chagas-Paula D.A., Oliveira R.B., da Silva V.C., Gobbo-Neto L., Gasparoto T.H., Campanelli A.P., Faccioli L.H. and Da Costa F.B., *J. Ethnopharmacol.*, 2011; **136(2)**: 355-362.

[9] Ejelonu O.C., Elekofehinti O.O. and Adanlawo I.G., *Biomed. Pharmacother.*, 2017; **87**: 589-595.

[10] Lu M.R., Huang H.L., Chiou W.F. and Huang R.L., *Pharmacogn. Mag.*, 2017; **13(52)**: 702-706.

[11] Ojo O.A., Ojo A.B., Ajiboye B.O., Olaiya O., Okesola M.A., Boligon A.A., de Campos M.M.A., Oyinloye B.E. and Kappo A.P., *Toxicol. Rep.*, 2018; **5**: 585-592.

[12] Pantoja Pulido K.D., Colmenares Dulcey A.J. and Isaza Martinez J.H., *Food Chem. Toxicol.*, 2017; **109(2)**: 1079-1085.

[13] Sinha A.K., *Anal. Biochem.*, 1972; **47(2)**: 389-394.

[14] Karim N. and Tangpong J., *J. Health Res.*, 2018; **32(5)**: 364-370.

[15] Kurutas E.B., *Nutr. J.*, 2016; **15(1)**: 71-71.

[16] Di Giacomo C., Vanella L., Sorrenti V., Santangelo R., Barbagallo I., Calabrese G., Genovese C., Mastrojeni S., Ragusa S. and Acquaviva R., *PLoS One.*, 2015; **10(4)**: 1-15.

[17] Acquaviva R., Di Giacomo C., Vanella L., Santangelo R., Sorrenti V., Barbagallo I., Genovese C., Mastrojeni S., Ragusa S. and Iauk L,. *Molecules*, 2013; **18**: 3241–3249.

[18] John-Dewole, O.O., Oni, S.O., *IOSR J. Pharm. Biol. Sci.*, 2013; **6**: 21-25.

[19] Karim N., Jeenduang N. and Tangpong J., *Walailak J. Sci. Technol.*, 2018b; **15(2)**: 107-116.

[20] Jung J.Y., Lim Y., Moon M.S., Kim J.Y. and Kwon O., *Nutr. Metab.*, 2011; **8(1)**: 1-8.

[21] Dewanjee S., Das A.K., Sahu R. and Gangopadhyay M., *Food Chem. Toxicol.*, 2009; **47(10)**: 2679-2685.

[22] Miura T., Nosaka K., Ishii H. and Ishida T., *Biol. Pharm. Bull.*, 2005; **28(11)**: 2152-2154.

[23] Fauziyah Y., Sunarti S., Hanoum I.F. and Wahyuningsih M.S.H., *Molekul*, 2018; **13(1)**: 72-79.

[24] Szkudelski T., *Physiol. Res.*, 2001; **50(6)**: 537-546.

[25] Matough, F.A., Budin, S.B., Hamid, Z.A., Alwahaibi, N., Mohamed, J., *Sultan Qaboos Univ. Med. J.*, 2012; **12(1)**: 5-18.

[26] Li S., Tan H.-Y., Wang N., Zhang Z.-J., Lao L., Wong C.-W. and Feng Y., *Int. J. Mol. Sci.*, 2015; **16(11)**: 26087-26124.

[27] Ayala A., Muñoz M.F. and Argüelles S., *Oxid. Med. Cell. Longev.*, 2014; 2014: 360438. DOI 10.1155/2014/360438.

[28] Maritim A.C., Sanders R.A., and Watkins J.B., *J. Biochem. Mol. Toxicol.*, 2003; **17(1)**: 24-38.

[29] Ighodaro O.M. and Akinloye O.A., *Alexandria J. Med.*, 2018; **54(4)**: 287-293.

[30] Zhang X.F. and Tan B.K., *Clin. Exp. Pharmacol. Physiol.*, 2000; **27(5-6)**: 358-363.

[31] Gawlik K., Naskalski J.W., Fedak D., Pawlica-Gosiewska D., Grudzień U., Dumnicka P., Malecki M.T. and Solnica B., *Oxid. Med. Cell. Longev.*, 2016; 2016: 2352361. DOI 10.1155/2016/2352361.

[32] Ben Salem M., Ben Abdallah Kolsi R., Dhouibi R., Ksouda K., Charfi S., Yaich M., Hammami S., Sahnoun Z., Zeghal K.M., Jamoussi K. and Affes H., *BMC Complem. Altern. Med.*, 2017; **17(1)**: 328-328.

[33] Kumarappan C.T., Thilagam E., Vijayakumar M. and Mandal S.C., *Indian J. Med. Res.*, 2012; **136(5)**: 815-821.

[34] Monday O.M. and Uzoma A.I., *Asian Pac. J. Trop. Biomed.*, 2013; **3(8)**: 628-633.

[35] Sarma R., Kumari S., Elancheran R., Deori M. and Devi, R., *Front. Pharmacol.*, 2016; **7**: 294-294.