



Antidiabetic and Antioxidant Effects of Acteoside from *Jacaranda mimosifolia* Family Biognoniaceae in Streptozotocin–Nicotinamide Induced Diabetes in Rats

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Abstract

BACKGROUND: Acteoside is a phenylethanoid compound isolated from *Jacaranda mimosifolia* D. Don leaves with a potential antidiabetic effect.

OBJECTIVES: This study was designed to investigate the antidiabetic and antioxidant effects of acteoside in streptozotocin-nicotinamide (STZ-NA)-induced Type 2 diabetes in rats.

METHODS: Diabetes was induced by intraperitoneal (i.p.) injection of a single dose of STZ (52.5 mg/kg), 15 min following i.p. administration of NA (25 mg/kg). Rats were divided into six groups; Group I: Normal rat group received the vehicle, Group II: Diabetic control group, and Groups III-IV: Diabetic rat groups were treated by either oral acteoside (10, 20, and 40 mg/kg) or pioglitazone (30 mg/kg) for 21 consecutive days. Biochemical parameters were assessed in the serum and liver homogenates. Examination of liver sections for histopathology was also carried out.

RESULTS: Acteoside treated rats showed significant lower levels of blood glucose, glycosylated hemoglobin, total cholesterol, triglycerides, and increased serum insulin compared to control diabetic rats. Furthermore, acteoside treated rats, in comparison to the diabetic control, demonstrated significantly reduced malondialdehyde, increased reduced glutathione liver contents, and attenuated pathological alterations in the liver. These effects were comparable to those caused by the standard antidiabetic drug, pioglitazone. *In vitro*, acteoside scavenged stable free radical 1,1-diphenyl-2-picrylhydrazyl.

CONCLUSION: Acteoside could be considered as a potential therapeutic agent for type 2 diabetes mellitus. However, studying further mechanisms underlying its antidiabetic effect is recommended.

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Introduction

Diabetes mellitus is a chronic metabolic disorder affecting about 450 million adults around the world. The number is expected to reach 629 million by 2045 [1]. The disease is characterized by hyperglycemia induced by diminished insulin output from pancreatic beta cells and/or tissues' resistance to insulin action. Type 2 diabetes is found in 90–95% of all diabetes cases. Diabetes is associated with high risk of macrovascular and microvascular (nephropathy, neuropathy, and retinopathy) complications [2]. Lifestyle modifications, pharmacological treatments and careful monitoring are the mainstay for diabetes management. Achieving target glycemic control helps preventing or at least can delay diabetes complications [3].

Despite the benefits of current antidiabetic drugs, every class has undesirable adverse effects. Therefore, searching for new treatments for type 2 diabetes is warranted. New drugs should be efficacious with minimal adverse effects and affordable cost. Diabetic patients use natural remedies that are thought

to improve glycemic control especially in areas where the cost of drugs imposes a real challenge [4]. The genus *Jacaranda* (Bignoniaceae) is found mainly in tropical and subtropical geographical areas. *Jacaranda mimosifolia* is native to Brazil but is also cultivated as an ornamental tree in Egypt. Acteoside (verbascoside) was isolated from the leaves of the *J. mimosifolia* [5]. A variety of promising activities of acteoside was reported in the previous studies including anti-inflammatory [6], [7], hepatoprotective [8], antioxidant [9], antineoplastic [10], and neuroprotective effects [11].

Previous reports have demonstrated the potential anti-hyperglycemic effect of acteoside. For example, acteoside prevented protein glycation *in vitro*, an activity that is correlated with antidiabetic drugs [12]. Moreover, plant extracts containing acteoside showed anti-hyperglycemic effects in experimental type 2 diabetes [13]. Furthermore, a more direct testing of the potential hypoglycemic action of acteoside was reported in the study by Morikawa *et al.* [14]; after 2 weeks of daily oral acteoside given concurrently with a starch load in mice, glucose tolerance was improved without significant change in weight. However, the

antidiabetic effect of acteoside has not been yet explored in experimental diabetes models. Therefore, this work aims to investigate the antidiabetic and antioxidant effects of acteoside in a rat model of Type 2 diabetes induced by streptozotocin-nicotinamide (STZ-NA).

Materials and Methods

Animals

Male Wistar albino rats (weight 180–210 g) were used in the current study. Rats were purchased from the Animal House Facility of the National Research Centre (Cairo, Egypt). Animals were housed in standardized conditions and allowed to acclimatize for 7 days in the laboratory before starting the experiment. Rats had free access to a standard food pellets and water *ad libitum*. The experimental protocol and all animal procedures were approved by the Ethics Committee of the National Research Centre, Egypt (approval number: 18/042). The committee guidelines are in line with the National Institutes of Health guide for the care and use of laboratory animals.

Materials

STZ was procured from Sigma-Aldrich (Missouri, USA), NA from Bayer Schering Pharma (Switzerland, Europe), and Pioglitazone from Amoun Pharmaceutical Industries Co., (Cairo, Egypt). Pure sample of acteoside was provided by the fourth author. Isolation of acteoside from *J. mimosifolia* leaves was carried out as previously reported by Moharram and Marzouk [5]. All other chemicals and reagents were of analytical grade.

Diabetes induction

After 12 h fasting, diabetes was induced in rats by a single intraperitoneal dose of STZ (52.5 mg/kg) dissolved in 0.1 mol/L citrate buffer (pH 4.5) [15]. STZ was given 15 min after intraperitoneal injection of NA (25 mg/kg) [16]. The next 24 h following STZ injection, a 5% glucose solution was given to rats to overcome the risk of death that may result from hypoglycemic shock. After 48 h of STZ injection, blood glucose levels were estimated in blood samples withdrawn from the tail vein using a portable glucometer. Rats were considered diabetic only if fasting blood glucose was ≥ 250 mg/dL.

Experimental design

After weighing rats, they were randomly distributed into six groups (six rats per group): Group I: Served as the normal control (given only distilled water);

Group II: Diabetic control group; Groups III-V: Diabetic rats treated with oral acteoside for 3 weeks (10, 20, and 40 mg/kg), respectively; and Group IV: Diabetic rats treated with oral pioglitazone (30 mg/kg) for 3 weeks. The vehicle was given to normal and diabetic control rats. Acteoside or pioglitazone treatments were started after confirmation of hyperglycemia, 48 h following STZ injection.

Acteoside and pioglitazone doses were selected according to the previously published data by Liu *et al.* [17] and by Vidal *et al.* [18], respectively.

Body weight changes

Initial body weight was determined by weighing each rat before the beginning of the experiment. Furthermore, the final body weight for each rat was estimated 24 h after the last dose of administration of either vehicle or treatment according to the study design. The percent change in body weight was calculated as follow:

$$\% \text{ Change in body weight} = (\text{Final body weight} - \text{initial body weight} / \text{Initial body weight}) \times 100$$

Biochemical analysis

Glucose level

Glucose level (mg/dl) was measured colorimetrically using kits purchased from (Biodiagnostic, Egypt) based on the method by Trinder [19].

Serum insulin level

Serum insulin level ($\mu\text{IU/ml}$) was measured by enzyme-linked immunosorbent kit Rat Insulin (INS) ELISA (Cusabio Biotech Co., Ltd., Hubei, China) following the manufacturer's protocol.

Glycosylated hemoglobin level (HbA1c)

Glycosylated Hb level (ng/ml) was estimated using (Rat [HbA1c] ELISA) purchased from Glory Science, following the manufacturer's protocol.

Serum triglyceride and total cholesterol levels

Triglycerides level (mg/dl) and total cholesterol level (mg/dl) were determined using enzymatic methods. Diagnostic kits from Biodiagnostic, Egypt, were used following the method of Fossati and Prencipe [20].

Preparation of tissue homogenate

All rats were sacrificed by decapitation under anesthesia, and then their livers were removed. A portion from the liver was homogenized in (20% w/v)

ice cold 0.1 M phosphate buffer (pH 7.4). Then, the homogenate was centrifuged at 4000 rpm for 5 min in a cooling centrifuge (2k15; Sigma/Laborzentrifugen). The supernatant was then used for determining the liver contents of malondialdehyde (MDA) and reduced glutathione (GSH).

Hepatic lipid peroxide content

Hepatic MDA content (nanomoles/gram of liver tissue) was determined colorimetrically, as described by Satoh [21] using a diagnostic kit purchased from BioDiagnostic Co., Egypt.

Hepatic GSH content

Hepatic reduced GSH content (mmol/g liver tissue) was estimated by a colorimetric method according to Beutler *et al.* [22] using a kit purchased from BioDiagnostic Co., Egypt.

Evaluation of the antioxidant effect (in vitro)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined following the method described by Peiwu *et al.* [23]. If the compound is antioxidant that can donate hydrogen, it will react with DPPH. The reaction will induce color change of DPPH from deep violet to yellow. This change in color was measured using a spectrophotometer at 517 nm. Ascorbic acid at 0.1 M concentration was the standard [24]. DPPH radical scavenging activity was calculated according to the equation:

$$\text{Radical scavenging activity \%} = \frac{(Ac - At)}{Ac} \times 100 \quad (1)$$

Where Ac and At are the absorbance of control (DPPH) and acteoside, respectively.

Histopathological examination

Liver tissues were taken from rats and fixed in 10% formaldehyde for 24 h. Then tissues were processed to obtain 4 μ m paraffin embedded sections. The tissue sections were stained by hematoxylin and eosin stain and examined using the light microscope [25].

Statistical analysis

Results are expressed as mean \pm SEM for six rats per group. Comparisons between more than two groups were carried out using one-way ANOVA followed by Tukey's multiple comparisons test. All analyses were done using GraphPad Prism 6.0 statistical package for Windows (GraphPad, San Diego, Calif.). Statistical significance was set at $p < 0.05$.

Results

Effect of acteoside on body weight

Figure 1 reveals that diabetes induced by a single i.p. dose of STZ (52.5 mg/kg) 15 min after the i.p. injection of NA (25 mg/kg) led to a significant body weight loss of percentage of body weight by 11.86 ± 1.32 after 3 weeks of diabetes induction. Meanwhile, normal rats showed a significant gain in % body weight by 22.74 ± 1.85 . Acteoside given orally to diabetic rats in doses of 10, 20, and 40 mg/kg for 21 successive days resulted in significant gain in percentage of body weight by 19.05 ± 1.14 , 16.92 ± 1.00 , and 22.98 ± 1.21 , respectively. Similarly, pioglitazone orally administered at 30 mg/kg showed a significant gain in percentage of body weight by 25.59 ± 2.13 .

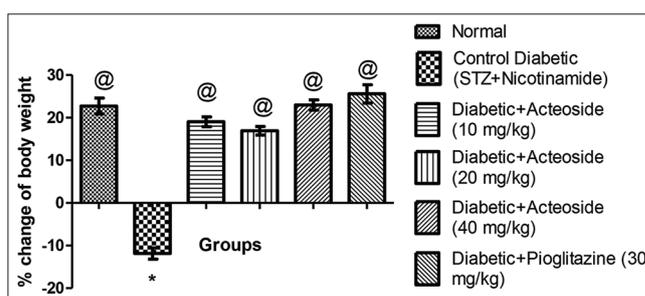


Figure 1: Effect of acteoside on body weight changes in diabetic rats. Results are expressed as % change of body weight ($n = 6$). Statistical analyses were carried out using one-way ANOVA followed by Tukey's multiple comparison test. *Significant difference from normal group at $p < 0.05$. @Significant difference from control diabetic group at $p < 0.05$.

Effect of acteoside on blood glucose, insulin levels, and HbA1c

As demonstrated in Table 1, diabetic rats showed a significant elevation in blood glucose to a level of 318.70 ± 13.8 mg/dl whereas normal rats mean level was 81.51 ± 4.15 mg/dl. Three weeks oral treatment with acteoside (10, 20, and 40 mg/kg) caused a significant reduction of blood glucose to 111.30 ± 0.61 , 74.88 ± 3.23 , and 75.15 ± 8.45 mg/dl, respectively, versus control value. Furthermore, pioglitazone (30 mg/kg) reduced blood glucose level to 103.00 ± 3.12 mg/dl versus control value of diabetic rats.

Regarding serum insulin levels, control diabetic rats showed a significantly decreased serum insulin level of 1.25 ± 0.07 μ IU/ml whereas normal rats 5.32 ± 0.27 μ IU/ml. Oral treatment with acteoside (10 mg/kg) resulted in significant elevation in serum insulin level to be 3.23 ± 0.06 μ IU/ml versus control diabetic and normal groups. Acteoside in dose of 20 mg/kg restored serum insulin level to be 5.38 ± 0.21 μ IU/ml versus control value of diabetic rats. Oral treatment with acteoside 40 mg/kg significantly increased serum insulin level to be 6.80 ± 0.20 μ IU/ml versus control diabetic and normal values. Pioglitazone treatment resulted in significant

increase in serum insulin level to be $8.00 \pm 0.16 \mu\text{U/ml}$ versus control diabetic and normal values (Table 1).

As depicted in Table 1, control diabetic rats had significantly elevated HbA1c level of $40.30 \pm 3.39 \text{ ng/ml}$ as compared to mean normal values of $3.36 \pm 0.21 \text{ ng/ml}$. Acteoside in doses of 10 and 20 mg/kg showed a significant elevation in HbA1c levels to be $24.12 \pm 1.88 \text{ ng/ml}$ and $16.72 \pm 1.06 \text{ ng/ml}$, respectively, versus values of control diabetic rats and normal rats. Acteoside (40 mg/kg) significantly reduced HbA1c level to be $10.18 \pm 0.92 \text{ ng/ml}$ versus control diabetic rats. Similarly, pioglitazone significantly reduced HbA1c level to be $6.42 \pm 0.29 \text{ ng/ml}$ versus values of control diabetic rats.

Effect of acteoside on total cholesterol and triglyceride levels

The effect of acteoside on total cholesterol and triglyceride is shown in Table 2. Control diabetic rats showed a significant increase in total cholesterol to be $139.35 \pm 4.28 \text{ mg/dl}$ as compared to normal values $94.38 \pm 2.38 \text{ mg/dl}$. Oral treatment with acteoside (10, 20, and 40 mg/kg) significantly reduced total cholesterol level to be 96.24 ± 1.08 , 90.62 ± 1.60 , and $95.14 \pm 4.65 \text{ mg/dl}$, respectively, versus control diabetic rats' values. In the same manner, pioglitazone (30 mg/kg) restored total cholesterol level to be $96.80 \pm 4.17 \text{ mg/dl}$ versus control value of diabetic rats. Concerning triglyceride levels, control diabetic rats showed a significant elevation in triglycerides level to be $153.90 \pm 3.33 \text{ mg/dl}$ as compared to normal values $107.80 \pm 2.00 \text{ mg/dl}$. Oral treatment with acteoside (10, 20, and 40 mg/kg) restored total cholesterol level to be 118.59 ± 6.46 , 100.27 ± 4.19 , and $91.89 \pm 3.20 \text{ mg/dl}$ versus control value of diabetic rats. Furthermore, pioglitazone restored triglyceride level to be $108.98 \pm 9.14 \text{ mg/dl}$ versus control diabetic rats' value.

Effect of acteoside on MDA and GSH in liver

Results depicted in Figure 2a show that control diabetic rats significantly elevated hepatic MDA content to be $65.27 \pm 2.63 \text{ nmol/g}$ as compared to normal rats $38.94 \pm 1.57 \text{ nmol/g}$. Oral treatment with acteoside (10, 20, and 40 mg/kg) restored hepatic MDA contents to be 32.63 ± 2.14 , 36.14 ± 2.75 , and $39.40 \pm 1.14 \text{ nmol/g}$, respectively, versus control diabetic rats' value.

Similarly, pioglitazone (30 mg/kg) restored hepatic MDA content to be $39.30 \pm 3.49 \text{ nmol/g}$ versus control diabetic rats' value.

Regarding hepatic GSH content, control diabetic rats significantly reduced hepatic GSH content to be $6.61 \pm 0.17 \text{ mmol/g}$ whereas normal values $8.69 \pm 0.16 \text{ mmol/g}$. Acteoside (10, 20, and 40 mg/kg) significantly increased hepatic GSH contents to be 8.99 ± 0.37 , 8.30 ± 0.21 , and $8.56 \pm 0.39 \text{ mmol/g}$ versus control diabetic rats' value. Furthermore, oral treatment with pioglitazone (30 mg/kg) restored hepatic GSH to be $9.00 \pm 0.30 \text{ mmol/g}$ versus control diabetic rats' value (Figure 2b).

In vitro antioxidant activity of acteoside

In vitro antioxidant activity of acteoside versus ascorbic acid (0.1 M concentration), using DPPH radical scavenging activity method is depicted in Figure 3. After reaction time of 5 min, different concentrations of acteoside (200, 150, 100, and 50 mg/ml) showed a maximum reactive reaction rates of 74.4, 74, 73, and 72.3%, respectively. In the same manner, the reactive reaction rate of L-ascorbic acid was 82.5%.

Effect of acteoside on histopathologic examination of the liver

In normal rats, no histopathologic alterations were found. Normal histological structure of the central vein and surrounding hepatocytes in the parenchyma was recorded in the normal group (Figure 4a). Pathological changes that recorded in the liver of control diabetic rats were severe dilatation and congestion of the central and portal vein associated with collagen proliferation and few inflammatory cells infiltration in the periductal tissue surrounding the hyperplastic bile ducts in the portal area (Figure 4b and c). Apoptosis was detected in few hepatocytes associated with diffuse kupffer cells proliferation (Figure 4d). Rats that were orally treated with acteoside (10, 20, and 40 mg/kg), respectively, showing marked reduction of the previously mentioned histopathological lesions (Figures 4e, f and 5a-d) that were observed in control diabetic group (Group II). Pioglitazone treated diabetic group (Group IV) showed few inflammatory cells infiltration in the portal area (Figure 5e). Furthermore, there was a diffuse kupffer cells proliferation in between the hepatocytes (Figure 5f).

Table 1: Effect of acteoside on blood glucose, serum insulin, and glycosylated hemoglobin in diabetic rats

Treatment	Parameters		
	Blood glucose (mg/dl)	Serum insulin ($\mu\text{U/ml}$)	GHbA _{1c} (ng/ml)
Normal	$81.51^{\text{a}} \pm 4.15$	$5.32^{\text{a}} \pm 0.27$	$3.36^{\text{a}} \pm 0.21$
Control diabetic (STZ+Nicotinamide)	$318.7^{\text{b}} \pm 13.8$	$1.25^{\text{b}} \pm 0.07$	$40.30^{\text{b}} \pm 3.39$
Diabetic+Acteoside (10 mg/kg)	$111.3^{\text{c}} \pm 0.61$	$3.23^{\text{c}} \pm 0.06$	$24.12^{\text{c}} \pm 1.88$
Diabetic+Acteoside (20 mg/kg)	$74.88^{\text{c}} \pm 3.23$	$5.38^{\text{c}} \pm 0.21$	$16.72^{\text{c}} \pm 1.06$
Diabetic+Acteoside (40 mg/kg)	$75.15^{\text{c}} \pm 8.45$	$6.80^{\text{c}} \pm 0.20$	$10.18^{\text{c}} \pm 0.92$
Diabetic+Pioglitazone (30 mg/kg)	$103.0^{\text{c}} \pm 3.12$	$8.00^{\text{c}} \pm 0.16$	$6.42^{\text{c}} \pm 0.29$

Results are expressed as mean \pm SEM (n=6). Statistical analyses were carried out using one-way ANOVA, followed by Tukey's multiple comparison test. ^aSignificant difference from normal group at $p < 0.05$. ^bSignificant difference from control diabetic group at $p < 0.05$.

Table 2: Effect of acteoside on total cholesterol and triglycerides in diabetic rats

Treatment	Parameters	
	Total cholesterol (mg/dl)	TG (mg/dl)
Normal	94.38 [±] 2.38	107.8 [±] 2.00
Control diabetic (STZ+Nicotinamide)	139.35 [±] 4.28	153.90 [±] 3.33
Diabetic+Acteoside (10 mg/kg)	96.24 [±] 1.08	118.59 [±] 6.46
Diabetic+Acteoside (20 mg/kg)	90.62 [±] 1.60	100.27 [±] 4.19
Diabetic+Acteoside (40 mg/kg)	95.14 [±] 4.65	91.89 [±] 3.20
Diabetic+Pioglitazone (30 mg/kg)	96.80 [±] 4.17	108.98 [±] 9.14

Results are expressed as mean±SEM (n=6). Statistical analyses were carried out using one-way ANOVA, followed by Tukey's multiple comparison test. *Significant difference from normal group at p<0.05. @Significant difference from control diabetic group at p<0.05. TG: Triglyceride.

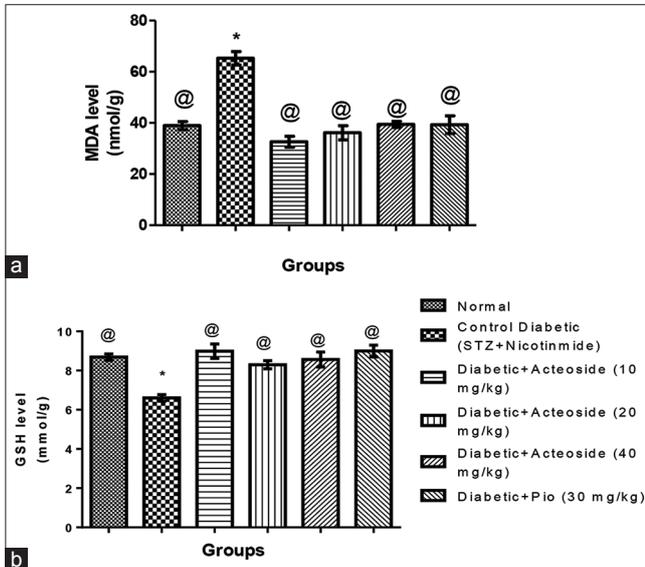


Figure 2: Effect of acteoside on (a) hepatic Malondialdehyde and (b) reduced glutathione contents in diabetic rats results is expressed as mean ± SEM (n = 6). Statistical analyses were carried out using one-way ANOVA followed by Tukey's multiple comparison test. *Significant difference from normal group at p < 0.05, @Significant difference from control diabetic group at p < 0.05

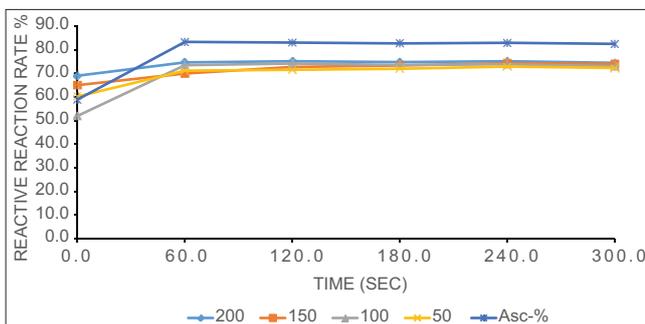


Figure 3: Antioxidant activity of acteoside and ascorbic acid (0.1 M concentration) in vitro, using 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity method

Discussion

Herbal medicines provide a valuable source of new antidiabetic therapies that could be safe and cost-effective [26]. Acteoside [(2R,3R,4R,5R,6R)-6-[2-(3,4-dihydroxyphenyl)ethoxy]-5-hydroxy-2-(hydroxymethyl)-4-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-3-yl] (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate) was isolated

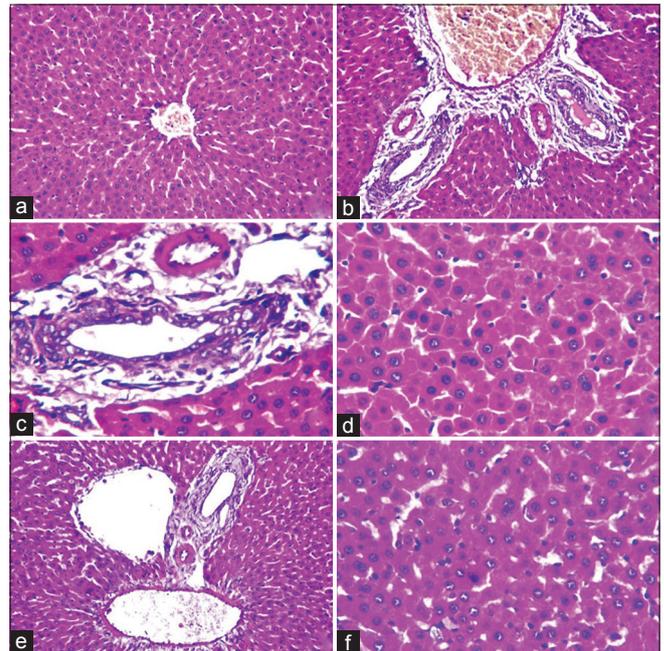


Figure 4: Photomicrograph of liver in different groups. (a) Group I showing normal histological structure of the central vein and surrounding hepatocytes (H&E, ×40). (b) Group II showing severe dilation and congestion of both central and portal veins with periductal collagen proliferation and few inflammatory cells infiltration surrounding the hyperplastic bile ducts in portal area (H&E ×40). (c) Higher magnification of the previous picture showing the congested portal and central vein with collagenous proliferation and few inflammatory cells infiltration surrounding the hyperplastic bile duct in portal area (H&E, ×40). (d) Group II showing the apoptosis in some few hepatocytes with diffuse kupffer cells proliferation in between H&E, ×80. (e) Group III showing dilatation of both central and portal vein with periductal collagen proliferation surrounding the bile duct at portal area (H&E, ×40). (f) Group III showing diffuse kupffer cells proliferation in between the hepatocytes (H&E, ×80)

from many medicinal plants and is becoming of interest because of its wide range of pharmacological actions [27]. Acteoside utilized in this work was isolated from *Jacaranda mimosifolia* D. leaves grown in Egypt. Hereby, we explored the antidiabetic effect of acteoside in a rat model of diabetes induced by STZ-NA. This animal model of Type 2 diabetes is considered a well-accepted experimental model that allows for preclinical examination of potential new antidiabetic agents [28]. In this work, pioglitazone was used for comparison. Pioglitazone belongs to the thiazolidinediones (TZDs) group of drugs which are used in the treatment of Type 2 diabetes. TZDs act as insulin sensitizers through activation of peroxisome proliferator-activated receptor-gamma (PPAR γ) receptors. Activation of these nuclear receptors affects glucose and lipid metabolism [29].

Results of the current work demonstrated significant antidiabetic and antioxidant effects of acteoside that was comparable to that of pioglitazone. Acteoside or pioglitazone treatment for 3 weeks caused a significant decrease in serum glucose and HbA1c as compared to their levels in control diabetic rats. This was associated with beneficial effect on body weight in contrast to the weight loss demonstrated in diabetic

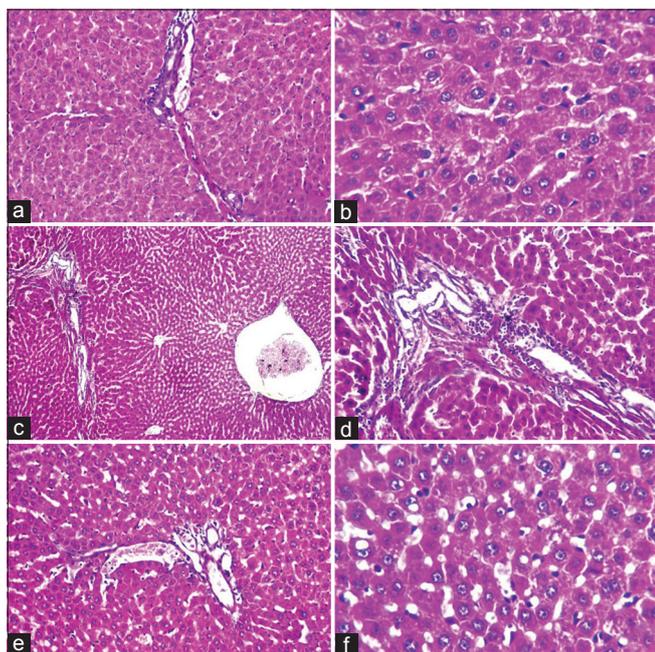


Figure 5: Photomicrograph of liver in different groups. (a) Group IV showing inflammatory cells infiltration in the portal area (H&E, $\times 40$). (b) Group IV showing diffuse kupffer cells infiltration proliferation in between the hepatocytes (H&E, $\times 80$). (c) Group V showing dilatation of the portal vein (H&E, $\times 16$). (d) Higher magnification of the previous picture showing the inflammatory cells infiltration in portal area (H&E, $\times 40$). (e) Group VI showing few inflammatory cells infiltration in portal area (H&E, $\times 40$). (f) Group VI showing diffuse kupffer cells proliferation in between hepatocytes (H&E, $\times 80$)

untreated group. Pioglitazone restored the reductions in rats' body weight caused by STZ-induced diabetes. This effect is reported by other studies and could be attributed to increase in insulin sensitivity which is a part of pioglitazone pharmacological actions [30]. However, in some reports, the drug was found to increase body weight due to increased appetite, lipogenesis, and fluid retention [31]. These different results in the literature could be related to the diabetes model used and the duration of the study. Improvement of body weight caused by acteoside could be related to its ability to reduce hyperglycemia. Other studies reported that acteoside reduces weight due to inhibition of pancreatic lipase [32] as well as improving levels of post prandial glucose in response to a load of starch in mice [14].

Our study demonstrated an antihyperglycemic effect of acteoside in STZ-NA-induced diabetes. Hyperglycemia increases the liability of proteins to glycation resulting in changes in their structure and function. Non-enzymatic glycation of globin fraction of hemoglobin produces HbA1c which is used for reliable monitoring of glycemic control in diabetes [33]. In agreement with the previous reports using STZ-NA model of diabetes; in this investigation, HbA1c was higher in the diabetic model group compared to the control vehicle group [34], [35]. Treatment with either pioglitazone or acteoside improved glycemic control over the study period as indicated by blood glucose and HbA1c levels in the treated groups. Pioglitazone

as a PPAR γ agonist improves fasting blood glucose and HA1c through increased insulin sensitivity in liver, muscle, and adipose tissue; effects were repeatedly reported both in experimental and in human diabetes [36], [37]. There was a comparable effect of acteoside on glycemic control in this study. This could be mediated through acteoside ability to scavenge free radicals produced by STZ with improvement of beta cell function and insulin levels or through improving target organ response to insulin. Acteoside successfully prevented *in vitro* production of advanced glycation end products (AGE) [12]. These products were found to induce resistance to insulin action in adipocytes, muscles, and hepatocytes [38]. In addition, high levels of AGE produced in chronic hyperglycemia bind to cellular membrane receptors to increase free radicals through activating NADPH oxidase [39]. Taking these studies into consideration, acteoside could have exerted its beneficial effect in diabetic rats, at least partly, through improving insulin resistance.

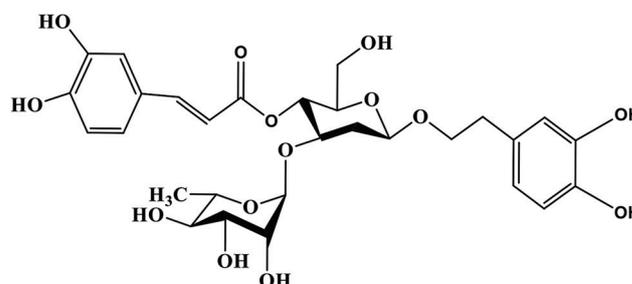
The findings of this study showed that 3 weeks treatment with pioglitazone or acteoside exerted a significant amelioration of total cholesterol and triglyceride (TG) levels, hepatic lipid peroxidation, and GSH. Moreover, it prevented the histopathological changes in liver architecture associated with STZ-NA diabetes. Reactive oxygen species production is augmented in diabetes and prolonged oxidative burden play a part in the development of long-term diabetes-related complications. Antidiabetic agents offering antioxidant action could be effective in preventing, or at least delaying, and progression of diabetes complications [40]. Pioglitazone antioxidant capacity is well documented in STZ models of diabetes and in various tissues including the liver [36], [41]. Structure-activity studies of acteoside revealed that the hydroxyphenyl ethyl and caffeoyl moieties of the compound are thought to be responsible for its antioxidant effects [27]. In the current experiment, to examine the antioxidant activity of acteoside in comparison to pioglitazone, hepatic MDA contents – an indicator of lipid peroxidation – and GSH liver contents were estimated. In addition, *in vitro* study using DPPH assay was employed to assess acteoside free radical scavenging activity. Acteoside showed antioxidant activity *in vitro* and was able to reduce MDA levels and restore GSH in livers of diabetic rats. Consistent with these results, previous studies reported the ability of acteoside to cause a significant reduction in lipid peroxidation and reversal of hepatic GSH depletion induced by carbon tetrachloride [8]. In a different study by Peerzada *et al.* [10], acteoside was able to protect rat liver cells against diethylnitrosamine-induced carcinogenesis by preventing DNA damage and cell apoptosis. The effect was further elucidated through acteoside's ability to scavenge reactive oxygen species produced by diethylnitrosamine. Furthermore, in a prior study, acteoside protected pulmonary endothelial cells against oxidative stress induced by hydroxyl radical. In this mentioned study, acteoside also demonstrated the

ability to scavenge DPPH radicals in a dose-dependent manner [42]. Studies investigating the antioxidant property of various phenylethanoids especially acteoside have demonstrated that these compounds protect cells due to their direct antioxidant and scavenging activity of free radicals. Other reports further explained the antioxidant effect of acteoside at subcellular levels. Acteoside upregulate the endogenous antioxidant-defensive systems [43]. This effect of acteoside could be exerted at the level of post-translational modification or at gene transcription of redox enzymes. *In vitro* and *in vivo* neuroprotective effects of acteoside was found to occur through activation of Nrf2, a transcription factor for genes encoding antioxidant and stress responsive proteins [9], [44].

Liver is one of the major organs affected by impaired insulin levels or action. In diabetes, there is activation of glycogenolysis leading to higher hepatic production of glucose. Furthermore, insulin resistance through changes in lipid metabolism and provision of inflammatory milieu contribute to the development of liver fatty changes. High levels of free fatty acids in insulin-resistance can exert direct toxic effect on hepatocytes and increase pro-inflammatory cytokines release contributing to hepatocellular changes seen in diabetes [45]. In our experiment, diabetic rats demonstrated higher levels of total cholesterol and triglycerides than the levels found in control non-diabetic rats. After 3 weeks daily administration of acteoside or pioglitazone, the levels of total cholesterol and triglycerides were improved in the diabetic group compared to their counterpart levels in diabetic untreated rats. In addition, histopathological examination of livers from diabetic rats showed histopathological changes that were significantly ameliorated by treatment with either pioglitazone or acteoside. This pioglitazone effect is in line with the previous reports. Pioglitazone ameliorated increases in total cholesterol and TG in STZ-induced diabetes; an effect that was accompanied by upregulation of hepatic PPAR γ [46]. Regarding the improvement in cholesterol and TG levels after treatment with acteoside it could be explained by its antidiabetic effect which was sustained throughout the study period as indicated by lower levels of HBA1c in treated rats. However, the literature provides evidence linking acteoside to PPAR- α (PPAR- α). PPAR- α is expressed in high amounts in liver, muscles, heart, and kidney, and acts chiefly to control genes concerned with lipids and lipoproteins metabolism. In the liver, this transcription factor control genes of proteins and enzymes regulating β -oxidation of fatty acids and lipid transport [47]. In a report by Esposito *et al.* [48], PPAR- α was linked to the anti-inflammatory effect of acteoside in experimental inflammatory bowel disease model of PPAR- α knock-out mice. Moreover, in a recent review of natural products with potential anti-dyslipidemic effect through targeting PPAR- α , acteoside was considered as one of these products [49]. However, more studies are needed to prove this mechanism of acteoside action.

Conclusion

The evidence from this study suggests that acteoside has antidiabetic and antioxidant effects. In addition, it offered advantages in the control of Type 2 diabetes induced by STZ-NA in rats through ameliorating body weight changes, cholesterol, and TG levels. The antioxidant effects were evidenced by reducing lipid peroxidation, DDPH activity and elevating GSH content as well as ameliorating hepatic histopathological alterations of diabetes. However, more in depth experimental studies of the detailed mechanisms behind these beneficial effects are warranted.



Structure of acteoside

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