ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 10, Issue 4, pp: (36-43), Month: October - December 2023, Available at: www.paperpublications.org

ANTIOXIDANT AND ANTI-HYPERLIPIDEMIC ACTIVITY OF AQUEOUS EXTRACT OF *Calotropis* procera STEM IN ALLOXAN-INDUCED DIABETIC RATS

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DOI: https://doi.org/10.5281/zenodo.10040483

Published Date: 25-October-2023

Abstract: **This study investigated the antioxidant and anti-hyperlipidemia of aqueous extract of *Calotropis procera* stem in alloxan-induced diabetic rats. Thirty (30) adult rats weighing between 100-200g were grouped into 6 groups with 5 animals in each group. Group I served as normal control, group II served as diabetic control, group III served as diabetic rats given metformin (Mtf 10mg/kg), group IV served as diabetic rats given 100mg/kg aqueous extract of *Calotropis procera* (AECp), group V serve as diabetic rats given 150mg/kg aqueous extract of *Calotropis procera* (AECp), group VI serve as diabetic rats given 200mg/kg aqueous extract of *Calotropis procera* (AECp). After administration of alloxan, the results revealed a significant increase (P<0.05) in the blood glucose levels and a decrease in the activities of antioxidants; CAT, SOD, GPx, and GSH with the corresponding significant increase in the activity of MDA compared to the normal control group. The result also showed a significant increase (P<0.05) in TG, TC, LDL, VLDL, and AI, with a significant decrease in HDL levels when compared to the normal control group. However, oral administration of (AECp) and metformin significantly decreased blood glucose levels in groups (III, IV, V and VI) and prevented the loss of body weight as compared to the diabetic control. AECp also protects the liver antioxidants against oxidative stress by alloxan used to induce diabetes mellitus. Hyperlipidemia was also restored in rats administered AECp as compared to the diabetic control group. The result revealed that the aqueous extract of *Calotropis procera* stem ameliorated the alloxan-induced hyperglycemia and hyperlipidemia in diabetic rats.**

Keywords: ***Calotropis procera*****, antioxidant, hyperlipidemia, diabetes mellitus.**

1. INTRODUCTION

Diabetes mellitus is a life-threatening metabolic disease that occurs when the pancreas cannot produce sufficient insulin or when it cannot effectively use the insulin produced thereby leading to hyperglycemia (American Diabetes Association 2010). Diabetes mellitus produces an onset of micro and macrovascular complications which progress to coma and death if unattended to. Diabetes mellitus reduced life expectancy, mortality, and morbidity in developed and developing countries. Diabetes mellitus presents symptoms such as weight loss, excessive urination, excessive thirst, excessive hunger, and blurred vision (Bearse *et al*., 2004). If unattended to may lead to an onset of progression of retinopathy, nephropathy and neuropathy; (Hove *et al*., 2004; Seki *et al*., 2004; Saely *et al*., 2004) and macrovascular disease (coronary artery, heart, and peripheral vascular diseases) Pittas AG (2009). Plants are valuable sources for the discovery of novel pharmacologically active compounds. This is because of the broader degree of chemical diversity and novelty of molecules found in natural products than that from any other source. Many drugs are derived directly or indirectly from plants (S.M.K 2001).

Calotropis procera is an evergreen perennial shrub that is widely distributed in low-water and semi-water environments. It is locally called "Bomu bomu" (Yoruba), "Tumifafiya" (Hausa) (Agbogidi *et al.*, 2006), "Otosi" in Igbo (Ajagbonna *et al*., 2019. *C. procera* has been used for the treatment of diseases such as skin disease, elephantiasis, toothache, asthma, leprosy, and rheumatism (Sharma *et al*., 2011). Different parts such as leaves, roots and bark, flower, fruits, stem, and latex of the plant have been reported to possess various phytochemicals that might possess various pharmacological activities. The coarse shrub possesses acaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, antidiarrheal, anticancerous, and larvicidal activities with other beneficial properties (Ahmed *et* al., 2005). This study aimed to investigate the hypoglycemic activity and toxicity of aqueous extract of *Calotropis procera* stem in alloxan-induced diabetic rats. Since diabetes mellitus leads to alterations in lipid metabolism, hypertriglyceridemia and hypercholesterolemia are the most common form of lipid abnormalities in diabetes mellitus.

2. MATERIALS AND METHODS

Glucometer (Accu-Check Advantage II glucometer; Roche Diagnostic Co, USA), Electronic weighing balance (OHAUS), Spectrophotometer (JENWAY model 7305), electrolyte analyzer, test tubes, Centrifuging machine, Syringes and Needles, Cotton wool, Plain tubes, EDTA tubes, Rotary evaporator (Bibby scientific limited stone, Staffordshire, ST15 OSA, UK) model no. RE300/MS, water bath (HH digital thermostatic water bath, model no. DK-420).

Chemicals/drugs

Alloxan (Sigma, St. Louis, Germany) was procured from Jos, Nigeria, Metformin was purchased from a reputable pharmaceutical store in Keffi, Nassarawa State, Nigeria. All chemicals used were of analytical grade and they were procured from Merck/Sigma Company.

Calotropis procera Sample

Calotropis procera plant was obtained from the premises of Nassarawa State University, Keffi. The stem bark of *Calotropis procera* was cut and washed with water to remove all contaminants. It was dried at room temperature and grounded to powder for four (4) weeks

Sample preparation

The powdered stem was extracted by aqueous solution by cold maceration method for 24 hours. The extract solution was filtered through Whatman filter paper (No. 1) and concentrated by evaporation using a rotary evaporator at a reduced pressure to recover the crude extract. The aqueous crude extract was stored in the refrigerator until needed for experiments.

Research Design

Thirty (30) adult rats of 100-200g body weight were induced with diabetes with an intraperitoneal (i.p.) injection of alloxan, 150 mg/kg per body weight which was dissolved in normal saline (NS). The animals were allowed free access to both food and water while they acclimatized to the environment for one week. On day 3, the Fasting blood glucose levels were measured and rats with blood glucose levels greater than 9.7 mmol/l (175 mg/dl) were included in the study (Yadav *et al*., 2002). The standard hypoglycemic drug (metformin) was administered to diabetic rats once daily at doses that were considered safe based on previous work (Sangraula *et al*., 2002). The rats were divided into six (6) groups, five (5) rats per group.

Group I: (normal control)

Group II: (diabetic control)

Group III: (diabetic rats given metformin (Mtf 10mg/kg)

Group IV: (diabetic rats given 100mg/kg AECp stem)

Group V: (diabetic rats given 150mg/kg AECp stem)

Group VI: (diabetic rats given 200mg/kg AECp stem)

The results were recorded and the blood samples were taken to the laboratory for further analysis. Blood glucose levels were recorded for days 0, 3, 7, 21 and 28. Rats were sacrificed after 28 days and the pancreas was removed for histology assays.

ISSN 2349-7823

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Phytochemical screening

Qualitative phytochemical screening was determined by the method described by Trease and Evans (1989) and Sofowora (1993).

Determination of blood glucose level

Blood glucose levels were determined based on the glucose oxidase method by Barham *et al.*, (1972). Blood samples of the rats were collected by cutting the tail of the rats for blood glucose determination before administering the extract. Administration of the extract commenced on day 3 after induction for a period of 28 days.

Lipid profile

In the plasma, triglyceride was determined using the method of Fossati and Prencipe 1982, HDL-C was determined with the method of LopezVirella *et al.*, 1977, and total cholesterol was determined using the method of Zollner and Kirsch 1962. LDL-C and VLDL-C were calculated from the obtained lipid parameters using the formula by Friedewald et al. 1972. Cholesterol ratio (CR) was calculated with the formula: Cholesterol ratio ¼ TC HDL − C Atherogenic index (AI) was calculated using the following formula: AI ¼ TC − HDL − C HDL – C.

Antioxidants

The liver antioxidants such as catalase activity (CAT) (1952), superoxide dismutase activity (SOD) (1987), reduced glutathione (GSH) (1959), glutathione-s-transferase (GST) (1974), lipid peroxidation (MDA) 1979

Histopathology

The histopathology of the tissues of the pancreas and liver of an alloxan-induced male Wistar rats was determined by Drury and Wallington (1980).

The tissues were processed histologically and the haematoxylin and eosin staining technique was employed, (Lillie, 1954).

Statistical analysis

All data were expressed as mean ± SD (n=5). The statistical significance was evaluated by one-way analysis of variance (ANOVA) and individual comparisons were obtained by Ducan's multiple range test (DMRT) using SPSS (v.20.0, Chicago USA) values were considered statistically significant when $p<0.05$.

3. RESULTS

The qualitative phytochemical screening of *Calotropis procera* stem.

The results of the qualitative phytochemical screening of *Calotropis procera* stem are presented in Table 1. The results showed the presence of phenols, tannins, flavonoids, terpenoids, steroids, alkaloids and saponin in the extract.

The quantitative phytochemical screening in the AECp stem.

The results of the quantitative phytochemical analysis of *Calotropis procera* are shown in Table 2. The results showed the concentration of phenols (10.17mg/g), tannins (5.39mg/g), flavonoids (21.00mg/g), terpenoids (7.23mg/g), steroids (89.67mg/g), alkaloids (2.87mg/g), saponins (57.22mg/g) respectively.

Table 1: Qualitative phytochemical screening of *Calotropis procera* stem

Phytochemicals	Present
Phenols	+
Tannins	+
Flavonoids	+
Terpenoids	+
Steroids	+
Alkaloids	+
Saponins	+

+ indicates present, - indicates absent

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Table 2: quantitative phytochemical in the stem of cp

Phytochemical	Conc. (mg/g)
Phenols	10.17
Tannins	5.39
Flavonoids	21.00
Terpenoids	7.23
Steroids	89.67
Alkaloids	2.87
Saponins	57.22

Effect of blood glucose concentration in normal and alloxan-induced diabetic rats.

The results of the blood glucose concentration are presented in Table 3. The results showed that glucose was 95.40±5.09mg/dl in the normal control group and significantly ($p<0.05$) increased to 341.62±8.31mg/dl in the diabetic control group. However, oral administration of AECp stem at 100, 150 and 200mg/kg body weight significantly ($p<0.05$) decreased to 165.86±14.55mg/dl, 131.16±6.61mg/dl, 113.76±38.20mg/dl) respectively. No significant difference ($p<0.05$) between the group V treated with 150mg/dl and the standard drug (metformin).

Table 3: Effect of AECp stem on blood glucose levels in alloxan-induced diabetic rats

Groups	Day0	Day3	Day7	Day14	Day28
NC	95.88±1.71	95.40±5.09 ^{a}	99.72±5.94 ^{a}	99.00±6.05 ^{a}	97.92±4.88 ^{a}
DC	89.94±3.09	341.62±8.31^{d}	335.26±15.31^{d}	345.50±11.48^{d}	341.56±5.60^{c}
Mtf	89.28±2.77	273.60±28.59 ^{b}	222.84±31.22^{c}	162.00±13.50^{a,b}	131.04±6.84 ^{a}
AECp 100mg/kg	89.28±4.71	304.20±32.10^{b,c}	222.84±17.29^{c}	190.74±18.66^{b,c}	165.86±14.55 $^{a.b}$
AECp 150mg/kg	89.64±2.44	246.20±10.32 ^{b}	190.16±10.62^{b}	143.08±8.79 ^{a,b}	131.16±6.61 ^{a}
AECp 200mg/kg	87.48±6.35	251.28±16.80 ^{b}	186.48±6.43 ^{b}	128.16±3.24 ^{a}	113.76±38.20^{a}

Data's are expressed as mean ± SEM, (n = 6),

Means with different superscripts are significantly different ($p<0.05$).

NC = normal control, DC = diabetic control, Mtf = metformin, AECp = aqueous extract of *Calotopis procera*.

Table 4: Effect of AECp stem on body weight (mg/kg) in alloxan-induced diabetic rats

Groups	Day0	Day7	Day14	Day21	Day28
NC	125.4±11.8	124.26±41 ^{d}	126.4±32.1 ^{d}	127.5±28.0 ^{d}	129.19±19.3 ^{d}
DC	121.2±30.12	119.2±22.5 ^{b,c}	116.3±13.8 ^{b,c}	113.8±34.6 ^{b,c}	113.61±21.4 ^{b,c}
Mtf	108.0±09.1	104.6±47.2^{a}	103.2±08.6 ^{a}	105.3±16.1 ^{a}	107.4±35.7 ^{a}
AECp 100mg/kg	119.6±43.4	114.2±25.3 ^{b}	113.9±15.0 ^{b}	114.6±22.8 ^{b}	116.1±33.5 ^{b}
AECp 150mg/kg	117.3±27.9	115.7±35.5 ^{b}	117.3±43.2 ^{b}	119.6±36.4 ^{b}	120.1±27.2 ^{b}
AECp 200mg/kg	113.0±31.6	111.7±17.1 ^{b}	112.1±40.8 ^{b}	115.4±24 ^{b}	116.5±33.0 ^{b}

Data's are expressed as mean ± SEM, (n = 6),

Means with different superscripts are significantly different ($p<0.05$).

NC = normal control, DC = diabetic control, Mtf = metformin, AECp = aqueous extract of *Calotopis procera*.

Effect of AECp stem on antioxidants in alloxan-induced diabetic rats

A significant ($p<0.05$) difference was observed in diabetic control groups as compared with normal control group. However, oral administration of AECp stem significantly ($p<0.05$) increased in rat-treated groups in a dose-dependent manner.

The effect of AECp stem in the liver tissue, and the concentration of MDA as a marker of lipid peroxidation was evaluated as shown in table 4. Oral administration of AECp stem significantly ($p<0.05$) decreased the levels of MDA in rats treated groups. No significant difference ($p<0.05$) between groups V, VI and metformin groups as compared with the normal control group.

ISSN 2349-7823

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Table 5: Effect of AECp stem on antioxidants in alloxan-induced diabetic rats

Groups	SOD (U/mg)	CAT (U/mg)	GPX (U/mg)	GSH (mg/L)	MDA (mg/L)
NC	312.69±0.92 ^{b}	94.57±1.80 ^{d}	792.14±24.57 ^{d}	51.62±1.28 ^{c}	0.39±0.78 ^{a}
DC	114..44±0.56 ^{d}	53.83±3.01 ^{a}	246.15±31.51 ^{a}	25.79±2.04 ^{a}	2.07±0.67 ^{d}
Mtf	233.54±20.17 ^{b}	81.64±4.77 ^{b}	630.43±7.20 ^{b,c}	31.49±1.76 ^{a}	0.39±0.65 ^{a}
AECp 100mg/kg	210.01±3.75 ^{b}	78.91±2.39 ^{b}	536.03±8.41 ^{b}	34.96±1.13 ^{a,b}	1.29±0.14 ^{c}
AECp 150mg/kg	212.81±0.69 ^{a}	93.55±2.80 ^{d}	562.35±13.05 ^{b}	64.00±1.93 ^{d}	0.66±0.07 ^{a}
AECp 200mg/kg	226.08±13.22 ^{b,c}	86.98±2.80 ^{b,c}	554.91±7.47 ^{b}	57.21±1.44 ^{c}	0.68±0.89 ^{a,b}

Data's are expressed as mean ± SEM, (n = 6),

Means with different superscripts are significantly different ($p<0.05$).

NC = normal control, DC = diabetic control, Mtf = metformin, AECp = aqueous extract of *Calotopis procera*.

Table 6: Effect of AECp stem on lipid profile in alloxan-induced diabetic rats

Groups	TG (mg/dl)	TC(mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI (mg/dl)
NC	95.80±1.50 ^{c}	73.20±1.71 ^{c}	61.20±1.36 ^{b}	7.32±1.73 ^{a}	19.32±0.45 ^{c}	0.15±0.02 ^{a}
DC	126.80±3.4^{d}	119.60±3.3^{e}	30.80±1.77 ^{a}	63.44±4.38 ^{e}	25.36±0.69 ^{d}	0.94±0.09 ^{c}
Mtf	94.00±2.51 ^{c}	87.40±1.99 ^{d}	62.00±2.65 ^{b}	7.68±2.54 ^{a}	18.80±0.50 ^{c}	0.58±0.06 ^{b}
100mg/kg	86.40±1.21 ^{c}	63.20±1.07 ^{b}	74.40±1.44 ^{c}	28.48±0.96 ^{c}	17.28±0.24 ^{c}	0.89±0.04 ^{c}
150mg/kg	62.60±1.6 ^{b}	62.40±0.93 ^{b}	78.00±1.58 ^{c}	16.12±1.76^{a,b}	12.52±0.32 ^{b}	0.61±0.05 ^{b}
200mg/kg	40.80±2.63 ^{a}	43.80±1.28 ^{a}	73.80±1.28 ^{c}	38.16±1.02 ^{d}	8.16±0.53 ^{a}	0.13±0.02 ^{a}

Data's are expressed as mean ± SEM, (n = 6),

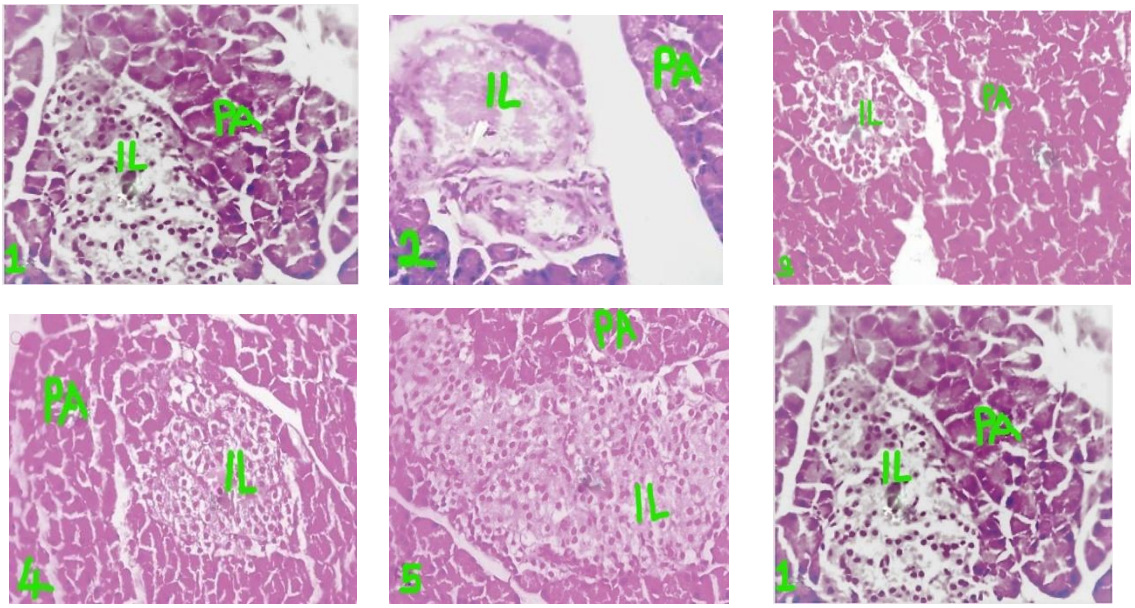
Means with different superscripts are significantly different ($p<0.05$).

NC = normal control, DC = diabetic control, Mtf = metformin, AECp = aqueous extract of *Calotopis procera*.

Effect of AECp stem on lipid profile in alloxan-induced diabetic rats.

Induction of alloxan significantly ($p<0.05$) increased the levels of TG, TC, LDL, and VLDL and decreased HDL, AI as shown in Table 6. However, administration of AECp stem significantly ($p<0.05$) decreased the levels of the lipid parameters above and increased HDL and AI above. The highest dose 200mg/kg body weight was more effective in lowering the levels of TG, and TC as compared to normal control groups. Also, no significant ($p<0.05$) difference in the normal control group, metformin and 100mg/kg body weight AECp groups.

Effect of AECp stem on the histology of pancreas in alloxan-induced diabetic rats



Histology of pancreas in alloxan-induced diabetic rats. **a**: (Normal control) pancreas showing prominent islet of Langerhans (IL) and pancreatic acini. **b**: (diabetic control) pancreas showing degenerated islet of Langerhans (IL) with no cells. **C**: (metformin) pancreas showing mild regeneration of islet of Langerhans (IL)and pancreatic acini (PA). **d:** (AECp 100mg/kg) pancreas showing regenerating islets (IL) and pancreatic acini (PA). **e:** (AECp 150mg/kg) pancreas showing prominent islet of Langerhans (IL) with numerous islet cells and PA. **f**: (AECp 200mg/kg) pancreas showing conspicuous islet of Langerhans (IL) and pancreatic acini (PA).

4. DISCUSSION

Diabetes mellitus is a chronic metabolic disease which is characterized by high glucose levels in the blood which can lead to diabetic complications and death if not treated.

Alloxan monohydrate is a laboratory chemical commonly used to induce diabetes mellitus in animals. Alloxan is mediated through selective inhibition of glucose-induced insulin secretion by glucokinase inhibition, the glucose sensor of the β-cells and it causes a state of insulin-dependent diabetic mellitus through its ability to induce ROS formation resulting in the selective necrosis of β-cells. Alloxan rather deposits in the pancreatic β-cells through a transporter via GLUT 2. This is established in the presence of intracellular thiols such as glutathione, in a cyclic redox reaction, alloxan generates ROS with dialuric acid (reduction product). This generates radicals through autoxidation such as superoxide, hydrogen peroxide and hydroxyl radicals being catalyzed by iron. This final step is responsible for the death of the β-cells which resulted in elevated glucose levels in the blood due to the insufficient secretion of insulin (Rohilla and Ali, 2012)

Phytochemical screening of *Calotropis procera* stem (table 2) indicates the presence of bioactive compounds such as tannins, phenol, terpenoids, alkaloids, saponins, flavonoids and steroids. The presence of these compounds might be responsible for the biological role of the plant which might be a measure as a therapeutic agent for the treatment of diabetic mellitus.

Induction of alloxan resulted in a significant ($p<0.05$) increase in the blood glucose levels (341.56±5.60) in diabetic control groups as compared with the normal control groups (97.92±4.88). However, oral administration of AECp once daily and metformin for a period of 28 days significantly ($p<0.05$) decreased the blood glucose levels 165.86±14.55, 131.16±6.61, 113.76±38.20) respectively as compared with the normal control groups which represent the reversal of the insulin resistant or secretion possibly by degeneration of pancreatic β-cells in alloxan-induced diabetic mellitus. The mechanism by which it exhibited this action might be due to the presence of flavonoids, saponins, and phenols which are some constituents of the AECp stem that acts as a free radical scavenger. The mechanism is because of the insulin-mimetic effect on the peripheral tissues by either stimulation of the regeneration process or release of the pancreatic secretion of insulin from existing β-cells.

One of the consequences of diabetes mellitus is weight loss there was a significant ($p<0.05$) decrease in the body weight of rats following the administration of alloxan, a decrease in body weight of the diabetic untreated group was due to dehydration and impaired carbohydrate, protein and fat metabolism (Chatterjea *et al*., 2002). However, oral administration of AECp significantly ($p<0.05$) increased the body weights of rat-treated groups as compared to diabetic untreated groups as a result of improvement in glycemic control (Voltarelli & de Mello 2008; Muthuraman et al. 2009; Abdel-Daim 2014; Yusuf et al. 2016).

Since diabetic mellitus leads to alterations in lipid metabolism, there was a significant change in the lipid profile. The most common lipid abnormalities in diabetes mellitus are hypertriglyceridemia and hypercholesterolemia. This is usually due to the increased metabolization of free fatty acids in the fat deposits. In this study, a significant ($p<0.05$) increase was noticed in TG, TC, LDL, VLDL, AI and decreased HDL, in diabetic control groups. However, administration of all the doses of AECp (100, 150 and 200mg/kg) body weight and metformin significantly ($p<0.05$) decreased the levels of TG, TC, LDL, VLDL, AI and increased HDL, this could be as a result of the presence of triterpenoids, tannins, phenols and this could possibly be of clinical significance by preventing diabetes mellitus complications such as coronary heart disease, atherosclerosis.

SOD, is a metalloprotein that is involved in antioxidant defense by scavenging superoxide radicals. CAT, a hematoprotein present in the peroxisome catalyzed the breakdown of hydrogen peroxide into H_2O and O_2. GPX also an antioxidant catalyzed the reaction between hydrogen-peroxide and disulfide. In this study, the levels of these antioxidants in the liver

were significantly ($p<0.05$) decreased in diabetic control groups as compared with normal control groups (El-Missiry *et* al., 2004). However, treatment with AECp stem and metformin significantly ($p<0.05$) increased these antioxidants which indicates a protective effect of the AECp stem on the liver. This action might be a result of the presence of flavonoids, alkaloids, and phenols. MDA, a marker for oxidative stress was significantly ($p<0.05$) increased in diabetic control groups as compared to normal control groups. Administration of AECp and metformin significantly ($p<0.05$) decreased MDA in a dose dependent manner.

In this study the histological assays indicate an improvement in treated groups as compared with diabetic control groups. The pancreatic β-cells showed degeneration and vacuolizations in the islet of Langerhans cells, decreased number of islets, islet shape and size (Hadi *et al*., 2016). Oral administration of AECp reversed the pancreatic β-cells back to normal

5. CONCLUSION

The aqueous extract of *Calotropis procera* stem ameliorated the alloxan-induced hyperglycemia and toxicity in diabetic rats. This was proven to be effective on the significant decrease of the elevated parameters which might have been resulted from synergistic effects of the bioactive components present in the *Calotropis procera* stem.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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