Original Article

Aristolochia ringens extract ameliorates oxidative stress and dyslipidaemia associated with streptozotocin-induced hyperglycaemia in rats

Abdulhakeem Olarewaju Sulyman[1], Jubril Olayinka Akolade[2], Raliat Abimbola Aladodo[1], Rasheed Bolaji Ibrahim[1], Asiat Na'Allah[3] and Azeemat Titilola Abdulazeez[3]

[1]Department of Biochemistry, Kwara State University, Malete. [2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, Abuja. [3]Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria

ABSTRACT

The study was designed to assess antioxidant and antidyslipidaemic effects of terpenoid-rich extract from the root of *Aristolochia ringens* V. Hyperglycemia-induced oxidative stress and dyslipidemia were established in rats by single intraperitoneal administration of 65 mg/kg bw streptozotocin. Based on therapeutic dose determined in previous study, streptozotocin-induced rats were orally administered with 75 and 150 mg/Kg bw of *A. ringens* extract for 14 days. Total protein, serum lipid profiles and biomarkers of oxidative stress in liver and kidney of the experimental rats were determined. Atherogenic and cardiovascular disease risk indices were computed. Streptozotocin-induced hyperglycaemia significantly ($p < 0.05$) decreased activities of superoxide dismutase, catalase and glutathione transferase as well as the amount of reduced glutathione in both tissues indicating oxidative stress induced kidney and liver injury due to glucotoxicity. In comparison to non-treated hyperglycaemic rats, activities of the antioxidant enzymes and concentration of glutathione-H were significantly ($p < 0.0001$) increased, whereas malondialdehyde was reduced in the tissues of rats treated with both 75 and 150 mg/Kg bw of the extract. The extract also caused significant ($p < 0.001$) reduction in elevated levels of total cholesterol, triglycerides and low density lipoprotein-cholesterol levels, whereas concentration of the attenuated high density lipoprotein-cholesterol was increased in serum of the treated rats. Reduced atherogenic and cardiac risk indices were projected for the *A. ringens* extract-treated groups. Results from this study showed that extract from *A. ringens* root was rich in terpenoids and may reduce risks of complications associated with hyperglycemia-induced oxidative stress and dyslipidemia.

Keywords *Aristolochia ringens*, diabetes, dyslipidemia, hyperglycaemia, oxidative stress

INTRODUCTION

Hyperglycemia induced-oxidative stress is a secondary complication associated with diabetes mellitus. Oxidative stress, which results from overwhelm and subsequent reduction in innate oxidative defenses with concomitant increase in free radical generation, may occur through abnormal increase in glycolysis; autooxidation of glucose and glycation of enzymatic protein; all of which are consequences of persistent hyperglycemia (Marfella et al., 2014). Oxidative stress-induced insulin resistance and lipid peroxidation may lead to dysfunction in the metabolism of lipids (dyslipidemia) and related atherogenic complications in diabetic patients (Tangvarasittichai, 2015). Thus, oxidative stress plays a crucial role in the development and progression of vascular disorders in diabetic conditions.

Superoxide anion is the primary oxidant formed by the reduction of molecular oxygen that may lead to secondary radicals, reactive nitrogen and oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical following dismutation or interaction with other reactive species. ROS generation (specifically superoxide) is considered the unifying link between the various metabolic dysfunction induced by hyperglycemia, hence normalizing levels of superoxide production and other ROS ameliorate cellular damage induced by the glucotoxicity by blocking glucose-induced activation of protein kinase C, formation of advanced glycation end-products and sorbitol accumulation (Nishikawa et al., 2000).

Generally, compounds with excellent antioxidant capacity are used to normalize ROS production. Medicinal plants are rich repository of antioxidant phytochemicals which are capable of modulating metabolic changes through their radical scavenging activities, thus playing beneficial roles in the prevention and management of hyperglycaemia-induced oxidative stress. *Aristolochia ringins* (Vahl) is one of the most prominent among the medicinal plant decoctions used by African Traditional Medicine Practitioners (TMPs) in the treatment oxidative stress mediated complications such as cancer, inflammation, hypertension, diabetes and diarrhea (Adeyemi et al., 2012; Aigbe et al., 2017; Akindele et al., 2015; Ruth et al., 2014; A. Sulyman et al., 2016). *A. ringens* is a perennial plant of the Aristolo-chiaceae family which comprises about 500 species (Huber, 1993). Previous study in our laboratory showed that the ethan-olic extract of *A. ringens* root was predominated by aristolone and the extract has glucose lowering effect in streptozotocin-induced diabetic rats (Sulyman et al., 2016). Aristolone is an unsaturated α,β – ketone with good antioxidant

capacity; in vitro free radical scavenging and lipid peroxidation inhibitory activities of aristolone-rich fraction from other plants have been reported (Marrelli et al., 2015; Silou et al.). However, there is no literature to the best of our knowledge on the antioxidant effects of aritsolone-rich extract in in vivo model or on hyperglycaemia-induced oxidative stress. Thus, this study was designed to evaluate the effect of aristolone-rich extract from *A. ringens* roots on streptozotocin-induced oxidative stress and dyslipid-aemia in Wistar rats

MATERIALS AND METHODS

Plant materials
A. ringens roots were collected from Idumota, Lagos, Nigeria. It was authenticated at the herbarium unit of the Botany Department, University of Lagos, Nigeria where a voucher specimen (LUH 6234) was deposited.

Chemicals and reagents
Streptozotocin, glutathione (SCBT, Heildeberg, Germany), epinephrine, 2,2-diphenyl-1-picrylhydrazyl, 1-chloro-2,4-dinitrobenzene, sucrose (Sigma Aldrich, St Louis, MO, USA), ethanol, trichloroacetic acid, thiobarbituric acid (JHD, China), sodium hydroxide, hydrogen peroxide, sodium dihydrogen phosphate and disodium hydrogen phosphate (BDH, Poole, England). All other chemicals and reagents used were of analytical and research grades.

Experimental animals
Wistar rats (*Rattus norvegicus*) weighing 128 ± 8.05 g were obtained from the animal house of Department of Biochemistry, Kwara State University Malete, Nigeria. They were acclimatized for 14 days to standard housing conditions. Water and rat pellets were provided *ad libitum*. The research adhered strictly and conforms to the Principles of Laboratory Animal Care (NIH Publication, No. 85-23) (Council, 2010).

Preparation of extract
Preparation of the extract was carried out as described by Sulyman et al. (A. Sulyman et al., 2016). The roots of *A. ringens* were dried and macerated; 100 g of the pulverized roots was soaked in ethanol (70 %; 1 L) for 24 h with intermittent shaking. The broth was filtered and concentrated to about 10 ml using the rotary evaporator connected to a vacuum pump and further dried at 40 °C in a dryer. The dried extract was labeled as ARE (aristolone-rich extract) in air-tight container and stored at under refrigeration at 4 °C.

Characterization of extract
Total terpenoid content was estimated following the procedure employed by Chang et al. (2012) with slight modifications Eucalyptol was used as standard in lieu of urosolic acid. Approximately 245 mg eucalyptol equivalent / g extract was quantified in the extract. Previous study in our laboratory using GC-MS analysis showed that Aristolone (90.2 %) predominate the extract (Sulyman et al., 2016). Presence of aristolone in the sample was confirmed using a UV-VIS scanning spectro-photometry. The peak λ was 239 nm, characteristic of the unsaturated α,β – ketone (Košťálová et al., 1991). The extract was reconstituted in distilled water containing 0.5 % dimethyl-sulphoxide to give a stock solution.

Induction of experimental hyperglycemia
RESULTS

The rats were subjected to 12 h fast, prior to the induction of hyperglycaemia. Streptozotocin (65 mg/Kg bw) freshly prepared in 0.01 M citrate buffer (pH 4.5) was intraperitoneally administered to rats (Abdulrazaq et al., 2012). Rats in the non-induced group were injected with 0.5 ml of the citrate buffer to serve as control. Fasting blood glucose (FBG) was determined prior to induction using a commercial glucometer (AccuChek active, Roche Diagnostic GMBH, Mannheim, Germany). Rats with blood glucose > 250 mg/dL after 48 h and sustained hyperglycemia for the next 72 h (5 days after STZ induction) were considered hyperglycaemic and used in the main study.

Animal grouping and extract administration
Experimental rats were completely randomized into six groups of five animals each (A-F).
- Group A (non-induced control) received 0.5 ml of distilled water.
- Group B (streptozotocin-induced and non-treated) received 0.5 ml of distilled water.
- Group C (streptozotocin-induced treated with standard drug) received 0.5 ml of 14.2 mg/Kg bw metformin
- Group D (streptozotocin-induced treated with extract) received 0.5 ml of 75 mg/Kg bw ARE
- Group E (streptozotocin-induced treated with extract) received 0.5 ml of 150 mg/Kg bw ARE

Treatments were orally administered, once a day, for 14 days. The therapeutic doses were adopted based on dose determination study that was previously reported (Sulyman et al., 2016).

Preparation of tissue homogenate
Under diethyl ether anesthesia, the rats were sacrificed, blood was collected in sample tubes, centrifuged at 3000 rpm for 10 min and the sera were separated. The sacrificed rats were quickly dissected; liver and kidney were isolated, weighed and homogenized with 20 % w/v ice-cold 0.25 M sucrose solution. The homogenate were centrifuged at 4000 rpm for 15 min. The supernatant were decanted and kept under refrigeration and used for analyses within 24 hr.

Determination of biochemical parameters
Serum total cholesterol, triglycerides and high density lipoprotein cholesterol (HDL-c) were determined by using commercially available diagnostic kits obtained from Randox Laboratories (UK). Low density lipoprotein (LDL-c) was calculated as described by Friedelwald et al. (Friedewald et al., 1972). Estimation of protein (Lowry et al., 1951), reduced glutathione (Ellman, 1959) and malondialdehyde (Draper et al., 1993) concentration using assay for thiobarbituric reactive species as well as activities of superoxide dismutase (Misra and Fridovich, 1972), catalase (Sinha, 1972) and glutathione transferase (Habig et al., 1974) were also determined.

Statistical analysis
Data are expressed as mean of five replicates ± standard deviation. Student's t-test was used to compare between control and test experiments, while one way analysis of variance followed by Turkey's post hoc test for multiple comparisons using Graph Pad prism version 5.02. Values were considered significant at $p < 0.05$ (Graph Pad software, San Diego, California, U.S.A).

Effect of *A. ringens* extract on antioxidant system of hyperglycaemic rats

The effect of ARE from *Aristolochia ringens* on activities of catalase, glutathione transferase and superoxide dismutase in the liver and kidney of streptozotocin-induced rats are shown in Figures 1-3. Activities of the antioxidant enzymes were significantly ($p < 0.05$) lower in the liver and kidney of following induction of hyperglycaemic with streptozotocin (group B) when compared to values determined in non-induced rats (group A).Administration of ARE significantly increased active-ties of catalase [$F_{(5,25)} = 1265$ for liver and $F_{(5,25)} = 873$ for kidney; $p < 0.0001$; Figure 1], glutathione transferase [$F_{(5,25)} = 108.7$ for liver and $F_{(5,25)} = 80.12$ for kidney; $p < 0.0001$; Figure 2] and superoxide dismutase [$F_{(5,25)} = 1498$ for liver and $F_{(5,25)}$

significantly higher in rats treated with 150 mg/Kg b. wt. of the extract (group D), whereas activities of glutathione transferase were not significantly different when compared to those treated with 75 mg/Kg b.wt of ARE (group E).

Figures 4 and 5 show concentration of reduced glutathione and malondialdehyde estimated in the liver and kidney of hyperglycaemic rats following oral administration of the root extract from *A. ringens*. The non-protein sulfhydryl metabolite was significantly ($p < 0.05$) reduced and the lipid peroxide was increased in non-treated hyperglycaemic rats when compared to values determined in normoglycaemic rats. Administration of either the oral antihyperglycaemic agent (14.2 mg/Kg b. wt. of metformin, group C) or *A. ringens* extracts to streptozoticin-indu
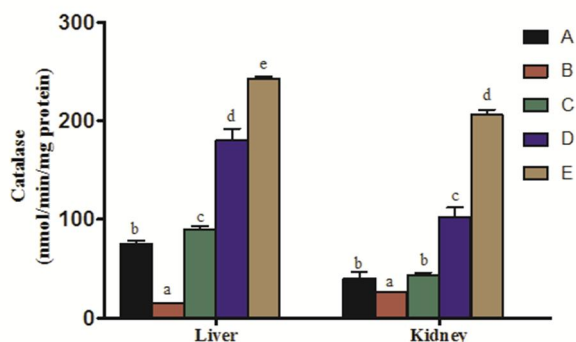

Fig. 1. Specific activity of catalase in the liver and kidney of streptozotocin-induced diabetic rats following oral administerat-ion of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different ($p < 0.05$). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract
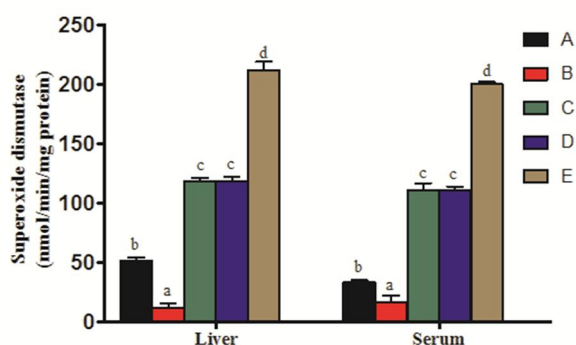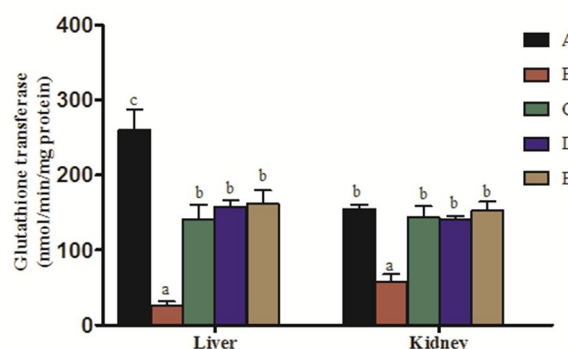

Fig. 2. Specific activity of glutathione transferase in the liver and kidney of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different ($p < 0.05$). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract

Fig. 3. Specific activity of superoxide dismutase in the liver and kidney of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different ($p < 0.05$). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract

= 1618 for kidney; $p < 0.0001$; Figure 3] when compared to those determined in the non-treated group (B). Activities of catalase and superoxide dismutase in both tissues were

Fig. 4. Concentration of reduced glutathione estimated in the liver and kidney of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different ($p < 0.05$). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract
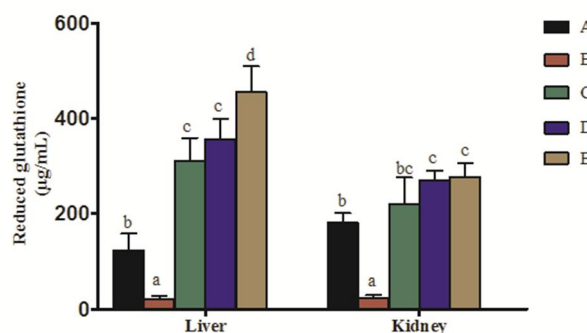
Table 1: Cardiac and artherogenic risk indices of streptozotocin-induced diabetic rats of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days

	Total Cholesterol/HDL-c	LDL-c/HDL-c
A	4.00	2.82
B	19.32	17.38
C	5.74	4.48
D	5.53	4.26
E	4.82	3.54

A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract

Fig. 5. Concentration of malonaldehyde estimated in liver and kidney of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different (p < 0.05). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract
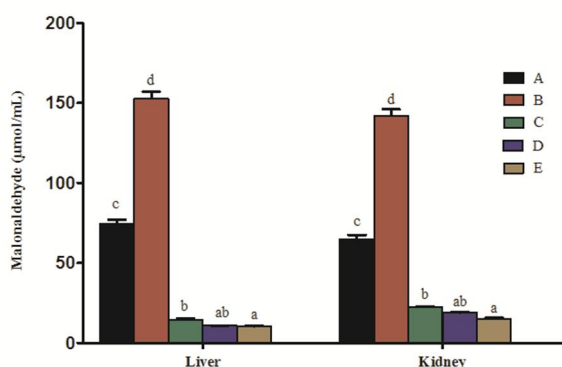
-ced rats significantly increased the concentration of reduced glutathione in both the liver [(F5,25) = 92.69; p < 0.0001)] and kidney [F(5,25) = 55.70; p < 0.0001] to ranges higher than values determined in non-induced rats. Conversely, levels of malondialdehyde in tissues of the treated rats were significantly [F(5,25) = 3778 and 3114 for liver and kidney respectively; p < 0.0001] lower than values estimated in non-treated rats.

Effect of *A. ringens* extract on serum lipid profiles of hyperglycaemic rats
Figure 6 shows the effect of administration of ARE on total cholesterol and triglycerides in the serum of hyperglycaemic rats. Induction of hyperglycaemia significantly (p < 0.05) increased the serum lipid when compared to values in normoglycaemic rats. Treatment of the rats with ARE significantly reduced concentration of the elevated serum total cholesterol [(F5,25) = 95.22; p < 0.0001)] and triglyceride [(F5,25) = 125.9; p < 0.0001]. Level of serum total cholesterol in rats treated with 150 mg/Kg b. wt. of the extract were significantly (p < 0.05) lower than those treated with 75 mg/Kg b. wt of the extract or metfor-min used as reference.
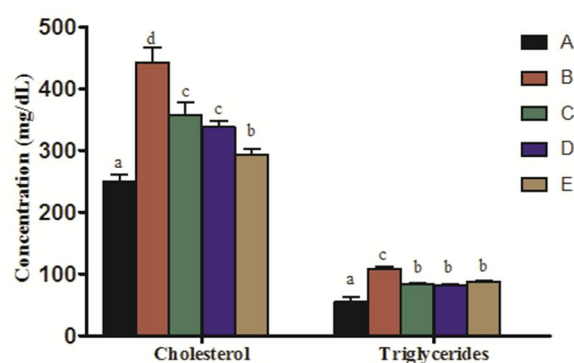

Fig. 6. Serum lipid profiles of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different (p < 0.05). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract

Fig. 7. Serum lipoprotein of streptozotocin-induced diabetic rats following oral administration of aristolone-rich extract of *Aristolochia ringens* for 14 days. Values are expressed as mean of five replicates ± SD and those with different superscripts across a row are statistically different (p < 0.05). A = non-induced control; B = non-treated induced control; C = induced treated with 14.2 mg/Kg bw metformin; D = induced treated with 75 mg/kg bw ARE; E = induced treated with 150 mg/Kg bw ARE; ARE = Aristolone-Rich Extract
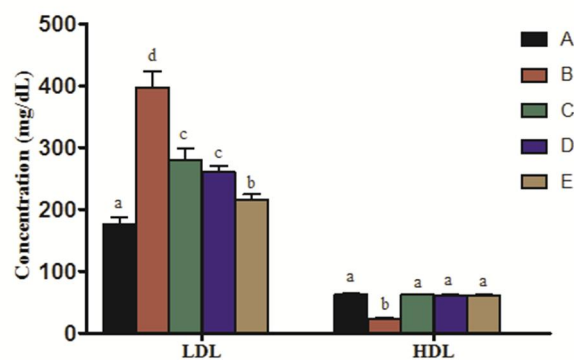
Concentration of serum lipoprotein fractions of streptozotocin-induced rats treated with *A. ringens* extract are

shown on Figure 7. HDL-c was significantly ($p < 0.05$) reduced and LDL-c was increased in streptozotocin-induced rats when compared to those of non-induced rats. Treatment of rats with the extracts significantly increased the concentration of HDL-c [$(F5,25) = 452.5$; $p < 0.0001$]] and reduced LDL-c [$(F5,25) = 125.3$; $p < 0.0001$]]. Cardiac and atherogenic risk indices were also reduced following treatment with the extract (Table 1).

DISCUSSION

A. ringens is commonly used by traditional medicine practition-ers for the treatment of various diseases such as haemorrhoids, asthma, diarrheal and diabetes in Nigeria. Anticancer, antidiarrheal, anti-inflammatory, antihypertensive, antitrypanos-omal, hepatoprotective and antidiabetic activities of the plant have been reported by various research studies (Adeyemi et al., 2012; Aigbe et al., 2017; Akindele et al., 2015; Osho and Lajide, 2014; Ruth et al., 2014; Sulyman et al., 2016; Sulyman et al., 2017). Previous study in our laboratory demonstrated the antidiabetic properties of *A. ringens* extract that is predominantly constituted by aristolone (Sulyman et al., 2016). The aristolone-rich extract significantly reduced hyper-glycaemia by inhibition of carbohydrate digesting enzyme (alpha-amylase) and modulation of other glucose metabolizing enzyme (hexokinase and glucose-6-phospahte dehydrogenase) resulting in increased utilization of glucose and conversion to glycogen in storage tissues.

Prolonged hyperglycaemia in diabetic conditions often results in vascular complications triggered by oxidative stress (Marfella et al., 2014). In this study, persisted elevation in glucose was observed following induction with streptozotocin. The antibiotic produced by *Streptomyces achromogenes* act as a diabetogenic agent by selectively destructing the pancreatic β-cells in rats and mice model via the action of reactive oxygen species (Szkudelski, 2001). The antioxidant defense system is compromised in streptozotocin-induced hyperglycaemic rats and the deleterious condition is characterized by alterations in activities of anti-oxidants enzymes as well as concentration of metabolites involved in cellular redox reactions (Szkudelski, 2012). An imbalance between production of reactive oxygen species and the antioxidant defense system may deregulate cellular functions leading to various pathological conditions (Bandyopadhyay et al., 1999).

Reduced glutathione is an intracellular reducing metabolite that protects cells against free radicals, peroxides, reactive oxygen and nitrogen species as well as other xenobiotics such as streptozotocin. Decreased concentration of the sulfhydryl metabolite observed following administration of streptozotocin may predispose the hyperglycaemic rats to complications associated with diabetes. The role of oxidized to reduced glutathione ratio as well as glutathione-dependent enzymes are crucial for thiol-sulfide exchange in maintaining cellular redox status, gene expression and activity of enzymes involved in such processes (Kalinina et al., 2014).

Treatment of the rats with the aristolone-rich extract caused elevation in the level of reduced glutathione via modulation of glutathione metabolism by stimulating the activity of glutathione transferase. Aristolone belongs to the terpene/terpenoid class of phytochemicals found in plants. Terpene and it derivatives are secondary metabolites that serve inherently as part of plant's defense system and are used as antioxidants to ameliorate oxidative stress (Grassmann et al., 2002). Terpenes are promi-sing agents in the prevention of diabetic complications resulting from oxidative stress such as advanced glycation end products that is implicated in

pathogenesis of diabetic nephropathy; aristolone-rich extract has been reported to cause significant increase in the level of glutathione-H in the kidney of hyperglycaemic rats indicating amelioration of kidney damage (Nazaruk and Borzym-Kluczyk, 2015).

Antioxidant activity of sesquiterpenoids including aristolone isolated from *Cyperus rotundus* was reported by Rani and Padmakumari (Rani and Padmakumari, 2012) and the antiproliferative activity of *Elionurus hensi* extract has been correlated with its percentage composition of aristolone (Silou et al.). Aristolone-rich extract from *A. ringens* did not only impr-ove the glutathione-dependent detoxification system but also increased the activities of other antioxidant enzymes such as catalase and superoxide dismutase. Superoxide dismutase is a ubiquitous enzyme that catalyzes the dismutation of superoxide radical to molecular oxygen and hydrogen peroxide, while catalase located mainly in the peroxisomes convert hydrogen peroxide to water and oxygen. Dysregulation of hepatic isoforms of these enzymes in streptozotocin-induced rats; amelioration was observed following treatment of the rats with a cocktail of natural antioxidants such as Vitamin C and E (Sindhu et al., 2004). Thus, the ameliorative effects of the *A. ringens* terpenoid-rich extract on oxidative stress in the diabetic rats may be attributed to the antioxidant capacity of aristolone. Antioxidant mode of action of the unsaturated α,β – ketone may be similar to other terpenoids such as carotenoids which involve quenching of singlet oxygen, hydrogen transfer or electron transfer (Grass mann, 2005).

In addition, the extract also ameliorated alterations in the lipid profiles of streptozotocin-induced diabetic rats. Streptozotocin is implicated in lipolysis (Szkudelski and Szkudelska, 2002) and lipid peroxidation (Armstrong and Al-Awadi, 1991). Estimated level of malondialdehyde; a biomarker of lipid hydroperoxide was significantly reduced in rats treated with *A. ringens* extract. Previous study by Marellli et al. (Marrelli et al., 2015) showed that extract from *Rosmarinus officinalis* containing appreciable amount of aristolone (11.3 % in the hexane fraction) exerted inhibitory effects on lipid peroxidation preventing the breakdown of linoleic acids to lipid hydroperoxides and further degradation to conjugated diene and unwanted volatile by-products.

Lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore, in addition to glycemic control, therapeutic regimes used in the management of diabetes, should have a favorable effect on lipid profiles. In correlation with other studies, elevated levels of total cholesterol, triglycerides and LDL-c with concomitant reduction in HDL-c were observed in the serum of streptozotocin-induced diabetic rats (Almuaigel et al., 2017; Kodikonda and Naik, 2017). Treatment of rats with terpenoid-rich extract significantly reduced the elevated levels of total cholesterol and triglycerides. The ratio of either total cholesterol or LDL-c to HDL-c was also reduced indicating lower predisposition of rats to atherosclerosis and cardiovascular diseases (Table 1). Although aristolone is a chemotypic compound native to *Aristolochia sp.*, it is also found in volatile/essential oil fraction of aromatic plants (Loumoua mou et al., 2017; Marrelli et al., 2015). Essential oils are derived mainly from the mevalonate pathway via condensation of isoprene units resulting in diverse array of terpenes and terpenoids. Medicinal role of essential oils in the treatment of cardiovascular diseases are well established. The antidyslipida-emic mode of action of *A. ringens* extract may be similar to those of other terpene-rich oils (Saljoughian et al., 2017), which may include inhibition of LDL-oxidation, up-regulation of LDL-receptor and down-regulation of sterol

regulatory element binding factors or proteins.

CONCLUSION

In conclusion, findings from this study showed that in addition to the capacity of extract from *A. ringens* to lower blood glucose (Sulyman et al., 2016), the extract also showed the capability to ameliorate oxidative stress and dyslipidaemia associated with diabetes. Thus, it is a promising bioactive candidate that can be further explored for management of diabetes and its associated complications.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors have no conflicting financial interests.

REFERENCES

Abdulrazaq NB, Cho MM., Win NN., Zaman R. and Rahman MT. Beneficial effects of ginger (Zingiber officinale) on carbohydrate metabolism in streptozotocin-induced diabetic rats. British Journal of Nutrition, 2012;108(7):1194-1201.

Adeyemi O, Aigbe F. and Badru O. The antidiarrhoeal activity of the aqueous root extract of Aristolochia ringens (Vahl.) Aristolochiaceae. Nigerian quarterly journal of hospital medicine, 2012;22(1):29-33.

Aigbe FR., Munavvar ASZ., Rathore H., Eseyin O, Pei YP, Akhtar S, Chohan A, Jin H, Khoo J, Tan S and et al. Alterations of haemodynamic parameters in spontaneously hypertensive rats by Aristolochia ringens Vahl. (Aristolochiaceae). Journal of Traditional and Complementary Medicine, 2018;8(1):72-80

Akindele AJ, Wani Z, Mahajan G, Sharma S, Aigbe FR, Satti N, Adeyemi OO and Mondhe DM. Anticancer activity of Aristolochia ringens Vahl. (Aristolochiaceae). Journal of Traditional and Complementary Medicine, 2015;5(1):35-41.

Almuaigel MF, Seif MA, Albuali HW, Alharbi O and Alhawash A. Hypoglycemic and hypolipidemic effects of aqueous extract of phaseolus vulgaris pods in streptozotocin-diabetic rats. Biomedicine & Pharmacotherapy, 2017;94:742-746.

Armstrong D and Al-Awadi F. Lipid peroxidation and retinopathy in streptozotocin-induced diabetes. Free Radical Biology and Medicine, 1991;11(4):433-436.

Bandyopadhyay U, Das D and Banerjee RK. Reactive oxygen species: Oxidative damage and pathogenesis. Current science, 1999;77(5): 658-666.

Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S and Hadley M. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. Free Radical Biology and Medicine, 1993;15(4):353-363.

Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Oates PJ and et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature, 2000; 404(6779):787-790.

Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics, 1959;82(1):70-77.

Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 1972;18(6):499-502.

Grassmann J. Terpenoids as plant antioxidants. Vitamins & Hormones, 2005;72:505-535.

Grassmann J, Hippeli S and Elstner EF. Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiology and Biochemistry, 2002;40(6):471-478.

Habig WH, Pabst MJ, Fleischner G, Gatmaitan Z, Arias IM and Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proceedings of the National Academy of Sciences, 1974;71(10):3879-3882.

Huber H. Aristolochiaceae. In: Kubitzki K., Rohwer J.G., Bittrich V. editors. Flowering Plants · Dicotyledons. The Families and Genera of Vascular Plants, vol 2. Springer, Berlin; 1993. p.129-137

Institute for Laboratory Animal Research(U.S.), National Academies Press (U.S.). Guide for the Care and Use of Laboratory Animals (8th edition). National Academies Press; 2010.

Kalinina E, Chernov N and Novichkova M. Role of glutathione, glutathione transferase, and glutaredoxin in regulation of redox-dependent processes. Biochemistry (Moscow), 2014; 79(13): 1562-1583.

Košťálová D, Hrochova V, Pronayova N and Leško J. Constituents of Aristolochia-clematitis L. Chemical Papers, 1991; 45(5):713-716.

Loumouamou AN, Bikindou K, Bitemou E, Chalard P, Silou T and Figueredo G. Optimization of the extraction of the p-menthadienol isomers and aristolone contained in the essential oil from Elyonurus hensii using a 23 full factorial design. Food Science & Nutrition, 2017;5(3):784-792.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 1951;193(1):265-275.

Madhuri K and Naik PR. Ameliorative effect of borneol, a natural bicyclic monoterpene against hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic Wistar rats. Biomedicine & Pharmacotherapy, 2017;96:336-347.

Marfella R, D'Onofrio N, Sirangelo I, Rizzo MR, Capoluongo MC, Servillo L, Paolisso G and Balestrieri ML. Polyphenols, Oxidative Stress, and Vascular Damage in Diabetes Diabetes: oxidative stress and dietary antioxidants. Academic Press; 2014.

Marrelli M, Cristaldi B, Menichini F and Conforti F. Inhibitory effects of wild dietary plants on lipid peroxidation and on the proliferation of human cancer cells. Food and Chemical Toxicology, 2015;86:16-24.

Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry, 1972;247(10):3170-5.

Nazaruk J and Borzym-Kluczyk M. The role of triterpenes in the management of diabetes mellitus and its complications. Phytochemistry Reviews, 2015;14(4):675-690.

Nishikawa T, Edelstein D, Du XL, Yamagishi Si, Matsumura T, Kaneda Y, Yorek MA, Nishikawa T, Osho I and Lajide L. Antitrypanosomal activity of Aristolochia ringens against Trypanosoma congolense infection in mice. Journal of Pharmacognosy and Phytotherapy. 2014;6(1): 1-3.

Rani MP and Padmakumari KP. HPTLC and reverse phase HPLC methods for the simultaneous quantification and in vitro screening of antioxidant potential of isolated sesquiterpenoids from the rhizomes of Cyperus rotundus. J Chromatogr B Analyt Technol Biomed Life Sci, 2012;904:22-28.

Ruth AF, Olaide, A. O. and Oluwatoyin, S. M. The aqueous root extract of Aristolochia ringens (Vahl.) Aristolochiaceae inhibits chemically-induced inflammation in rodents. Pak. J. Pharm. Sci, 2014;27(6), 1885-1889.

Saljoughian S, Roohinejad S, Bekhit AE-DA., Greiner R, Omidizadeh A, Nikmaram N and Mousavi Khaneghah A. The effects of food essential oils on cardiovascular diseases: A review. Critical Reviews in Food Science and Nutrition, 2018;58(10)1688-1705.

Silou T, Nienzy JP, Nsikabaka S, Loumouamou AN and Bikindou K. Extraction of Elionurus Hensii K. Schum Essential Oil in a Domestic Scale in the "Plateau Des Cataractes" (Congo-Brazzaville). International Journal of Engineering Science Technologies, 2017;2(1).

Sindhu RK, Koo JR, Roberts CK and Vaziri ND. Dysregulation of hepatic superoxide dismutase, catalase and glutathione peroxidase in diabetes: response to insulin and antioxidant therapies. Clinical and experimental hypertension, 2004;26(1):43-53.

Sinha AK. Colorimetric assay of catalase. Analytical biochemistry, 1972;47(2):389-394.

Sulyman A, Akolade J, Sabiu S, Aladodo R and Muritala H. Antidiabetic potentials of ethanolic extract of Aristolochia ringens (Vahl.) roots. Journal of ethnopharmacology, 2016;182:122-128.

Sulyman AO, Akolade JO, Na'Allah A, Aladodo RA and Jamiu HO. Effect of Administration of Root Ethanolic Extract of Aristolochia Ringens on the Liver Functional Indices of Male Wistar Rats. Iranian Journal of Toxicology, 2017;11(1):55-58.

Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological research, 2001;50(6):537-546.

Szkudelski T. Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Experimental Biology and Medicine, 2012;237(5):481-490.

Szkudelski T and Szkudelska K. Streptozotocin induces lipolysis in rat adipocytes in vitro. Physiological research, 2002;51(3):255-260.

Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World journal of diabetes, 2015;6(3):456.

7