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Glucose lowering and pancreato-protective effects of *Abrus Precatorius* (L.) leaf extract in normoglycemic and STZ/Nicotinamide – Induced diabetic rats

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ABSTRACT

Ethnopharmacological relevance: *Abrus precatorius* (L.) leaves are used as folk medicine by the local communities in the western region of Ghana to treat diabetes mellitus; however, this health claim remains unverified scientifically.

Objective: The study investigated glucose lowering and pancreato-protective effects of *Abrus precatorius* leaf extract (APLE) in normoglycemic and STZ/nicotinamide (NIC)-induced diabetic rats.

Method: after preparation of APLE, it was subjected to phytochemical screening, proximate composition and elemental assessments by using standard methods. Oral glucose tolerance test (OGTT) and maltose, lactose and sucrose oral challenge were assessed in normoglycemic rats post-APLE. Morphological characteristics of glucose response curve (time of glucose peak and shape of glucose response curve) were determined. Subsequently, diabetes mellitus was experimentally established in normoglycaemic adult Sprague-Dawley rats (weighing 150–250 g) of both sexes by sequential injection of Streptozotocin (STZ, 60 mg/kg *ip*)-reconstituted in sodium citrate buffer and NIC (110 mg/kg *ip*)-reconstituted in normal saline (1:1 v/v) for 16 weeks. Except control rats (normal saline 5 ml/kg *ip;* baseline fasting blood glucose [FBG] of 6.48 mmol/L), rats having FBG (stable at 11.1 mmol/L or ≥ 250 mg/dL) 3 days post-STZ/NIC injection were randomly re-assigned to one of the following groups: model (STZ/NIC-induced diabetic rats), APLE (100, 200 and 400 mg/kg respectively *po*) and metformin (300 mg/kg *po*) and treated daily for 28 days. Bodyweight and FBG were measured on weekly basis. FBG was measured by using standard glucometers. On day 28, rats were sacrificed under chloroform anesthesia, blood collected via cardiac puncture; kidney, liver and pancreas surgically harvested. While the pancreas was processed, sectioned and H&E-stained for histological examination, fresh kidney and liver were homogenized for assessment of total anti-oxidant capacity. Median cross-sectional area of pancreatic islets of Langerhans was determined for each group by using Amscope.

Results: Cumulatively, APLE (100, 200 and 400 mg/kg respectively) dose-dependently decreased the initial FBG by 55.22, 76.15 and 77.77% respectively compared to model (−1.04%) and metformin (72.29%) groups. APLE treatment recovered damaged pancreatic β-cells and also increased median cross-sectional area ($x10^6$ μm^2) of pancreatic islets compared to that of model group. APLE significantly ($P < 0.05$) increased total anti-oxidant capacity (5.21 ± 0.02 AscAE μg/mL) of plasma, kidney and liver compared to model (4.06 ± 0.04 AscAE μg/mL) and metformin (4.87 ± 0.03 AscAE μg/mL) groups.

Conclusion: APLE has demonstrated glucose lowering and pancreato-protective effects in rats and arrested the characteristic loss in bodyweight associated with diabetes mellitus. This finding preliminarily confirms folk use of APLE as an anti-diabetic herbal medicine, whiles providing a rationale for further translational studies on APLE.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose (hyperglycemia) along with high risk of long term micro-vascular complications secondary to defects in either insulin secretion (type 1 DM) or insulin action (type 2 DM). Global prevalence of DM is over 9% in adults, which implies almost 347 million people are suffering from DM (Danaei et al., 2011). Diabetes mellitus is associated with risk of long-term complications; typically these complications may develop after many years (10–20 years) (Chen et al., 2011; Zheng et al., 2018). The common long-term complications are related to vascular damage doubling the risk of cardiovascular disorders (Strain and Paldánius, 2018). For example, about 75% of deaths among diabetics were attributed to coronary artery disease, stroke, and peripheral vascular disease (O'Gara et al., 2013). The primary micro-vascular complications of DM include damage to the retina (diabetic retinopathy), kidneys (diabetic nephropathy), and nerves (diabetic neuropathy) (Strain and Paldánius, 2018). Also, proximal diabetic neuropathy causes painful muscle wasting and weakness which in most cases lead to development of diabetic foot ulcers, a condition that may require amputation (Bolton, 2018). Further, initial studies seem to suggest a link between cognitive deficit and DM. It was reported that diabetics have a 1.2 to 1.5 fold decline in cognitive function compared to non-diabetics (Biessels and Despa, 2018). From a pathological stand point, both type 1 and type 2 DM are respectively caused by extensive destruction of pancreatic β-cells (as a result of production of T-cell like auto-antibodies) and insulin resistance (insensitivity of insulin receptors in insulin-responsive tissues to endogenous insulin) (Solomon et al., 2008). Although genetic predisposition is a key risk factor for DM, however, the environment (life style, diet, obesity and other non-specific factors) also represents a key risk factor, as DM concordance in identical twins is shown to be low (Hyttinen et al., 2003). Just like type 1 DM, type 2 DM has genetic predisposition but life style particularly eating pattern can increase the risk of type 2 DM (Lehtovirta et al., 2010). The most at risk are the middle-aged adults, although this pattern has recently changed, with younger adults now reported to be the most at risk (Pinhas-Hamiel and Zeitler, 2005a, b). Treatment of type 1 and type 2 DM has respectively being insulin-replacement therapy and the use of oral hypoglycemic agents such as sulfonylureas, metformin, acarbose, sodium-glucose co-transporter type 2 (SGLT-2) and dipeptidyl peptidase 4 (DPP-4) inhibitors just to mention but a sample. Despite availability of these diverse anti-diabetic therapies, their cost and side effects upon long term use tend to be counter-productive (Dujic et al., 2016; Holman et al., 2017; Riser Taylor and Harris, 2013; Santos et al., 2017), perhaps it is for this reason many indigenous tribes across tropical regions of the world, particularly Africa have renewed their reliance on their ethno-botanical resources not only as a means to manage common diseases including DM but also to avoid high cost of conventional medical care.

Native African herbal medicine practice involves ingestion or topical application of crude herbal preparations or concoctions prepared from a whole plant, specific plant part/s, or a combination of different plant parts by using water or alcohol, and it is normally prescribed by a 'medicine man', or suggested by a friend, family member or as handed down through oral tradition over many generations. These crude herbal preparations may contain many bioactive secondary plant metabolites and are used by all groups of people including special populations such as pregnant mothers (Kamatenesi-Mugisha and Oryem-Origa, 2007; Veale et al., 1992). The ethno-botanical contexts in which these crude herbal preparations are used normally reflect the belief systems and experiences of the people as well as the diversity of the ethnobotanical heritage. Many medicinal herbs are being enrolled as part of herbal medicine-based anti-diabetic therapy by many indigenous communities (Farzaei et al., 2017; Yadav et al., 2017) and these may also hold prospects as natural templates for pharmaceutical semi-synthesis of other related drugs (Boye et al., 2020). For example, a number of medicinal plants used in the traditional management of DM in Nigeria are reviewed (Ezuruike and Prieto, 2014). It is strongly advocated that indigenous healing systems, particularly herbal medicine be incorporated into the primary healthcare systems of countries naturally endowed with diverse ethnobotanical heritage provided that such herb-based therapies are scientifically validated (Popović et al., 2016; Tandon and Yadav, 2017). Unfortunately, *Abrus precatorius* (L.) enjoys extensive use in folk medicine of many human cultures; however, some of its ethnomedicinal claims by some human cultures remain scientifically unverified.

A case of interest is the use of *Abrus precatorius* leaves by local communities in the western region of Ghana as an anti-diabetic herbal medicine. As far as verifiable, no study has confirmed or otherwise this ethnomedicinal claim on *Abrus precatorius* leaves with respect to the local communities in Ghana. Unlike Ghana, a preliminary study has confirmed the use of *Abrus precatorius* seeds in traditional management of DM in Nigeria (Ezuruike and Prieto, 2014). Aside Ghana and Nigeria, many tribes from other regions of the world have used *Abrus precatorius* in diverse ways as part of their indigenous healing systems to improve human health (Reddy Palvai et al., 2014; Umamahesh and Veeresham, 2016).

In view of the ethnobotanical relevance and pantropic nature of *Abrus precatorius*, many studies have investigated some of its pharmacological properties as a means to explain and validate health claims on it. Some of these pharmacological investigations include sperm anti-motility (Ratnasooriya et al., 1991), immuno-modulatory (Tilwari et al., 2011), anti-inflammatory (Dhoble and Majumdar, 2014), anti-asthmatic (Taur et al., 2017), anti-trypanosomal (Hata et al., 2014), nephroprotective (Falayi et al., 2018), anti-oxidant (Reddy Palvai et al., 2014), anti-α-amylase (Yonemoto et al., 2014), anti-proliferative (Gul et al., 2013), anti-bacterial and anti-fungal (Mobin et al., 2018), and anti-helminthic (Reddy Palvai et al., 2014) effects just to mention but a sample.

Since the pharmacological properties of *Abrus precatorius* are attributed to its constituent bioactive secondary plant metabolites many studies sought to characterize the active constituents in order to shed light on their chemistry. For example, the seeds of *Abrus precatorius* contains one of the most poisonous plant alkaloids known as abrin (Dhoble and Majumdar, 2014; Menezes et al., 2007) necessitating detoxification of the seeds prior to use in phytomedicine (Maurya et al., 2015). Other active agents isolated and characterized from *Abrus precatorius* include agglutinin protein (Behera et al., 2016), triterpene (Yonemoto et al., 2014), cycloartane-type saponins (Stavrianidi et al., 2018), propylated flavonoids and a lignan (Li et al., 2019), serine protease (Serge et al., 2017), β-amylase (Sagu et al., 2015), isoflavan quinones (Hata et al., 2013) just to mention but a sample.

The present study is a response to verify the use of *Abrus precatorius* leaves by local communities in the western region of Ghana as an anti-diabetic herbal medicine. Specifically, the study investigated glucose lowering and pancreato-protective effects of *Abrus precatorius* leaf extract (APLE) by using oral glucose tolerance test (OGTT), maltose, lactose and sucrose (MLS) oral challenge and Streptozotocin (STZ)/Nicotinamide-induced diabetic rat model. Results from the present study are not only interesting but also could stimulate further scientific discourse on the topic.

2. Materials and methods

2.1. Chemicals and drugs

Acarbose (LGM Pharma, USA), Streptozotocin (Sigma-Aldrich, Milwaukee, USA), Metformin (Harman Finochem, India), Nicotinamide (Finar India Ltd); Glucose, maltose, and lactose (Shanghai Bojing Chemical Co., Ltd); sucrose (MilliporeSigma); H&E stain (Sigma-Aldrich). All other chemicals and reagents used for extraction and phytochemical assessment were of analytical grade.

2.2. Collection, identification and authentication of Abrus precatorius

Leaves of *A. precatorius* were collected from Sefwi Asawinso in the Bibiani-Ahwiaso-Bekwai District of the Western Region of Ghana in January 2017. Cross-identification with vernacular names of *Abrus precatorius* was done before identification and authentication by a qualified taxonomist at the herbaria section, School of Biological Sciences, University of Cape Coast, Ghana, where a voucher specimen (CC3366) was deposited.

2.3. Preparation of Abrus precatorius leaf extract (APLE)

Preparation of APLE was carried out as previously described (Umamahesh and Veeresham, 2016) with modifications. Briefly, the leaves of *A. precatorius* were hand-picked from the stems and dried under shade until they were fully dried. The dried leaves were milled into fine powder. The powdered leaf was then stored in airtight plastic containers whiles pending further preparations. One hundred and 20 g (120 g) of the powdered leaf was soaked in 700 mL of ethanol for 48 h and covered with cheesecloth after pre-treatments (Zhao et al., 2020). The content was stirred four times each day. At the end of the second day, the content was filtered by using a Buchner funnel. The ethanol was retrieved by using a rotary evaporator on a water bath leaving a dark-green syrupy liquid extract. The dark-green syrupy extract was then dried in a desiccator for over a month until it was completely dried. The yield of the dried final extract was determined and named *Abrus precatorius* leaf extract (APLE). APLE was stored in a desiccator until use. The procedure for the preparation of APLE was repeated several times in order to obtain enough APLE for all intended experiments.

2.4. Qualitative phytochemical analysis on APLE

Phytochemical analysis was conducted on APLE by using standard qualitative phytochemical methods as extensively described elsewhere (Hussain et al., 2018). Alkaloids, total phenols, saponins, tannins, glycosides, anthraquinones, flavonoids and triterpenes were phytochemically screened for by using phytocompound-specific confirmatory tests. Results from the phytochemical screening were indicated either as present (+) or absent (−).

2.5. Elemental analysis on APLE

Elemental analysis on APLE was determined according to standard methods (Method 3015A, US Environmental Protection Agency, 2008) as described previously (Lytle et al., 2008). APLE was subjected to acid oxidation (sulphuric acid-hydrogen peroxide digestion) to completely remove all organic matter (Zhao et al., 2017) prior to the elemental analysis. Similarly, blank digestion (digestion of the digestion mixture without sample) was carried out. After APLE digestion, samples were transferred quantitatively into 100 mL volumetric flasks and made up to volume.

2.5.1. Determination of calcium and magnesium

Calcium and magnesium were determined by EDTA titration. Briefly, an aliquot of digested APLE solution (10 mL) was pipetted into a 250 ml conical flask and distilled water (150 mL) added. A I mL each of potassium cyanide, hydroxyl-amine hydrochloride, potassium ferrocyanide and triethanol amine were added. A 20 mL of 10% sodium hydroxide was added to adjust pH upward and then 10 drops of calcon indicator was added to the solution and titrated against EDTA (0.005M) solution.

2.5.2. Determination of sodium, potassium, iron, zinc and phosphorus

To determine Na, K, Fe, Zn and P, respectively, an aliquot of digested APLE (2 mL) solution was pipette into a 25 ml volumetric flask. A 2 mL of blank digest was also added to a 2 mL standard (Na, K, Fe, Zn and P) solution to give it the same background as the digest. Distilled water (10 mL) was added to the standards as well as the APLE solution. A 4 mL of reagent B made up of ascorbic acid and reagent A were added to the standard and APLE solution. Distilled water was added to the volumetric flask to make up to a volume of 25 mL and allowed to stand for about 15 min for color development. After color development, the absorbance of the standard and APLE solutions were determined using a spectrophotometer at a wavelength of 882 nm. A standard curve was plotted using their concentrations against their respective absorbance. The concentration of Na, K, Fe, Zn and P were extrapolated from their respective standard curves using the formula:

$$\text{P/Na/K/Fe/Cu/Zn (ug/g)} = \frac{\text{C x solution volume;}}{\text{Sample weight (g)}}$$

Where C is the concentration of analyte from standard curve.

All determinations were done in triplicates.

2.6. Proximate composition analyses on APLE

The following proximate compositions (moisture content, ash, crude protein, fat, fibre, and carbohydrate) were determined from APLE per standard methods of the Association of Official Analytical Chemists (A.O.A.C) (Lay et al., 2014).

2.6.1. Determination of moisture content

To determine moisture content in APLE, porcelain crucibles were washed dried and weighed. APLE (5 g) was placed in the crucible and weighed. The crucibles containing the APLE were placed in an oven at a temperature of 105 °C for 48 h. At the end of the period, the crucibles were removed, cooled in a desiccator and weighed. The moisture content was then calculated as a percentage of moisture lost from the APLE by the formula:

$$\text{Moisture content} = \frac{W2 - W3}{W} \times 100 = \frac{\textit{weight of moisture}}{\textit{weight of sample}} \times 100$$

Where;

w_1 = weight of empty crucible.
w_2 = weight of crucible and sample before drying.
w_3 = weight of sample after drying to a constant weight.

2.6.2. Determination of ash content

Ash content was determined by placing a known amount of APLE in a crucible, weighed, and transferred to a hot plate charred over a period for smoke to go out. The charred samples were then transferred into a muffle furnace and ignited at 550 °C for 5 h. The crucible containing the samples were then cooled in desiccator and weighed. The percentage ash was estimated as: $\text{Ash content} = \frac{w_3 - w_1}{w_2 - w_1} \times 100$; Where;

w_1 = weight of empty crucible.
w_2 = weight of crucible + weight of sample.
w_3 = weight of crucible + weight of sample after ashing

2.6.3. Determination of crude protein

Crude protein was determined by weighting APLE (0.2 g) into a numbered kjedahl digestion flask. A 4.5 mL of digestion mixture was added and the sample was digested at 360 °C for 2 h. The digest was allowed to cool and diluted to 50 mL with distilled water. A 20 mL of the digest was immediately distilled after adding 10 mL of alkali mixture using boric acid (5 mL) as indicator. A 50 mL of the distillate was collected and titrated against HCl (0.00712M) to pink. Percentage protein was calculated using the formula:

$$\%\ N = \frac{(Sample\ Titre - Blank\ \ Titre) \times Molarity\ of\ HCl \times 14.007 \times 100}{APLE\ (mg)}$$

2.6.4. Determination of fat content

To determine fat content in APLE, a known amount of APLE was weighted into a 50 × 10 mm soxhlet extraction thimble. This was transferred into a 50 ml capacity Soxhlet extractor. A dried clean 250 mL round bottom flask was weighted. About 150 mL of petroleum spirit of boiling point 40-60 °C was added and connected to a soxhlet extractor; and extraction commenced for 5–6 h. After 6 h the flask was removed, cooled in a desiccator and weighed. The percentage fat/oil was calculated using the formula: Crude Fat (%) = $\frac{W\ (g)}{APLE\ (g)} \times 100$Where; W is weight of oil (g).

2.6.5. Determination of crude fibre

To determine crude fibre, APLE (0.5 g) was weighed into a boiling flask; 100 mL of 1.25% sulphuric acid solution was added and boiled for 30 min. Filtration was carried out in a numbered sintered glass crucible. The residue was transferred into a boiling flask and 100 mL of 1.25% sodium hydroxide solution added and boiled for 30 min. Filtration continues after the boiling and residue washed with water and methanol. The crucible was dried in an oven overnight at 105 °C and weighed. The weighed crucible was placed in a furnace at 500 °C for 3 h. The crucible was slowly cooled and weighed. Crude fibre was determined using the formula:

$$\%\text{Crude fibre} = \frac{Weight\ loss\ through\ ashing}{Original\ weight\ of\ APLE\ (g)} \times 100$$

2.6.6. Determination of Carbohydrate Content

To determine carbohydrate content, APLE was weighed into a 50 mL conical flask and 30 mL of distilled water added. The content was allowed to simmer gently on a hot plate for 2 h. It was topped periodically to 30 mL and allowed to cool after 2 h. The solution was filtered into a 50 mL conical flask and topped to volume. The APLE sample was kept for color development. A 2 mL of glucose standard solution and APLE sample were pipette into a set of boiling tubes, anthrone (10 mL) solution added to the boiling tubes mixed thoroughly and cooled under running tap water. The tubes were placed in a beaker containing boiling water in a dark fume cupboard for 10 min. The tubes were allowed to cool in cold water in the dark. The optical density (OD) of the standard and the APLE sample were measured at 625 nm using a spectrophotometer. A calibration curve was prepared from the standards and used to estimate glucose (mg) in the APLE aliquot using the formula:

$$\%\text{Carbohydrate} = \frac{C\ (mg) \times Extract\ volume}{Aliquot \times APLE\ (g)} \times 100$$

Where; C (mg) = carbohydrate concentration determined from the standard curve.

2.7. Acquisition and care of experimental animals

Healthy adult (7–8 weeks old) Sprague-Dawley rats of both sexes weighing between 150 - 250 g were used in the study. Rats were housed in stainless aluminium cages (34 cm × 47 cm × 18 cm) with wood shavings as bedding, fed with normal rodent pelleted chow (GAFCO, Tema, Ghana), and had access to water *ad libitum*, however, these conditions were varied to suit the specific requirements of some experiments. Rats were maintained under normal ambient laboratory conditions of temperature, humidity and 12 h dark - light cycle. Rats were allowed to acclimatize to conditions in the animal house of the Department of Biomedical Sciences for two weeks before commencement of all animal experiments.

2.8. Ethical clearance

All animal protocols in the study were reviewed and approved by the University of Cape Coast Institutional Review Board (Approval ID: UCCIRB/CHAS/2017/53). Also, national (guidelines on use of animals in scientific experimentation subscribed by Ghana) and international (National Institutes of Health, NIH, OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals. Rockville, MD: NIH/Office for Protection from Research Risks, 1996. WWW:http://grants.nih.gov/grants/olaw/olaw.htm) guidelines on human care and use of animals in scientific experimentation were strictly adhered to.

2.9. Dose selection

Selection of drug (APLE, Acarbose, Glucose, Metformin, Nicotinamide and Streptozotocin) doses for the study was based on both trial experiments and previous studies (Al Shamsi et al., 2004; Hamza et al., 2018; Umamahesh and Veeresham, 2016).

2.10. Oral glucose tolerance test (OGTT) in normoglycemic rats

OGTT was determined as per a previous study (Umamahesh and Veeresham, 2016) with modification. Briefly, rats were fasted overnight, fasting blood glucose (FBG) measured using standard glucose peroxidase method as previously described (Hall and Keuler, 2009), and then rats were assigned to one of six groups (Fig. 1). After 1 h (in the case of APLE) or 30 min (in the case of Acarbose and metformin) pre-treatments, rats in respective groups were orally administered with glucose (2 g/kg). Blood samples were obtained by tail amputation method (Sørensen et al., 2019) and blood glucose was measured at 0, 30, 60, 90, 120, 150, and 180 min post-glucose oral challenge by using a glucometer (Accu-Check® Active) and glucose test strips.

2.11. Maltose, lactose, and sucrose (MLS) oral challenge in normoglycemic rats

MLS oral challenge in rats was assessed according to a previous study (Toma et al., 2015) with modification. Briefly, rats were fasted overnight, fasting blood glucose (FBG) measured using standard glucose peroxidase method (Hall and Keuler, 2009), and then rats were assigned to one of six groups (Fig. 1). After oral exposure of rats in respective groups to either APLE (100, 200, and 400 mg/kg *po*) or Metformin (300 mg/kg *po*) for 1 h and 30 min respectively, rats were orally administered with maltose (4 g/kg *po*), lactose (4 g/kg *po*) and/or sucrose (4 g/kg *po*) respectively in separate experiments. Post-prandial blood glucose was monitored at 0, 30, 60, 90, 120, 150, and 180 min by using a glucometer (Accu-Chek® Active). Control rats received normal saline (5 ml/kg *po*) before MLS oral challenge.

2.12. Estimation of glycated hemoglobin

Estimation of glycated hemoglobin was calculated from average post-prandial blood glucose using a previously described method (Nathan et al., 2008; Wei et al., 2014). After measurement of average post-prandial blood glucose (APBC) for respective groups, glycated hemoglobin was estimated by using the formula: $AG\left(\frac{MMOl}{L}\right) = 1.5944\ X\ AIC - 2.5944$ (Nathan et al., 2008).Where, AG or APBC is the average post-prandial glucose concentration, AIC (HbA1c) is glycated hemoglobin expressed in percentage (%).

2.13. Establishment of experimental diabetes mellitus in normoglycemic rats using STZ and nicotinamide

Experimental Type 2 diabetes mellitus was established in overnight-fasted normoglycemic adult Sprague-Dawley rats of both sexes as

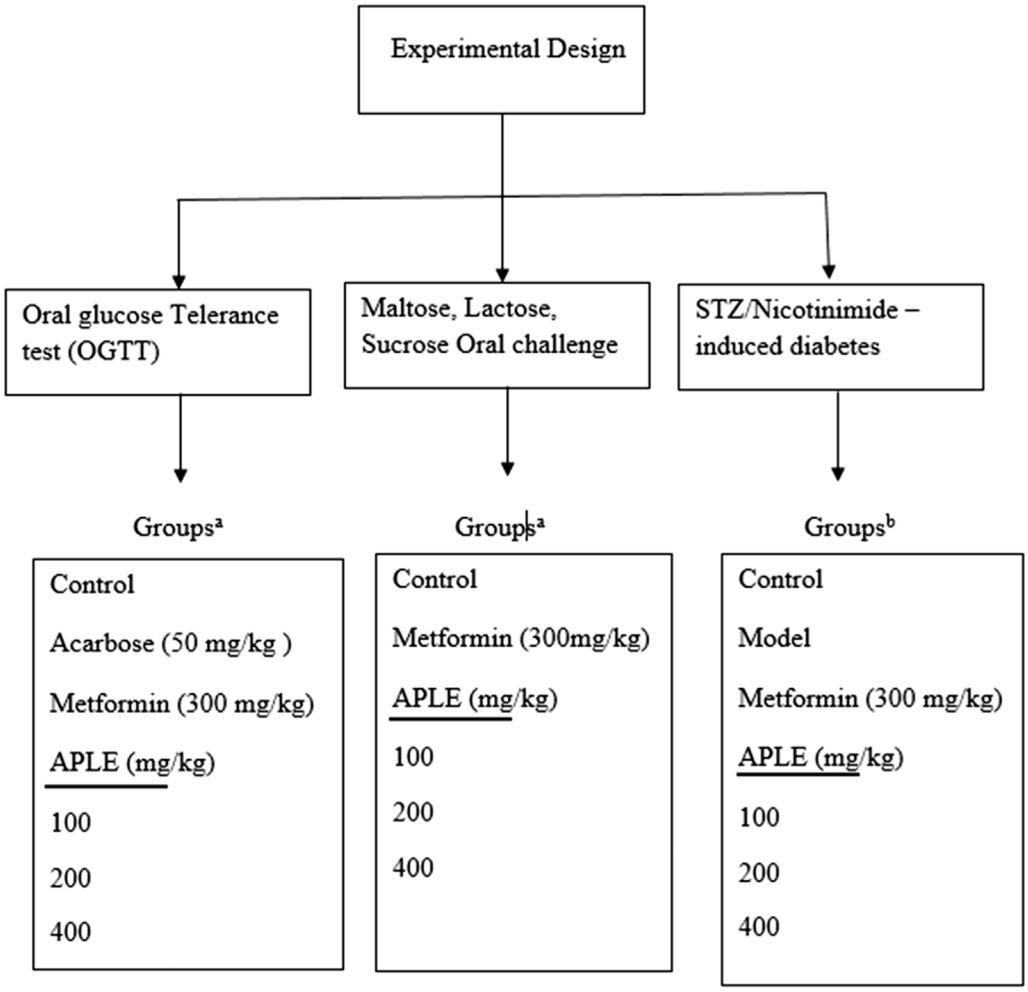

Fig. 1. An illustration of the experimental design. [a] Rats were pre-treated with respective drugs 1 h (in the case of APLE) or 30 min (in the case of Acarbose and Metformin) before oral administration of glucose (2 g/kg) or MLS (4 g/kg), then prandial blood glucose monitored every 30 min for 2 h [b] confirmed hyperglycemic rats (STZ/Nicotinamide exposure, FBG of 11.1 mmol/L or ≥ 250 mg/dL over 3 days) were randomly re-assigned to the various groups, then treated daily with the various drugs, then FBG monitored weekly in the morning for 28 days. APLE – *Abrus precatorius* leaf extract; FBG – Fasting blood glucose; MLS – Maltose, Lactose, and Sucrose; STZ – Streptozotocin.

previously described (Hamza et al., 2018) with slight modifications. Briefly, rats were sequentially injected Nicotinamide (110 mg/kg, *ip*) dissolved in normal saline and maintained on ice, followed 15 min later by injection of STZ (60 mg/kg, *ip*)-reconstituted in sodium citrate buffer solution (100 mM, pH 4.5). Subsequently, rats were orally administered 5% glucose (5 ml/kg) solution daily for 7 days. To confirm induction of hyperglycemia in rats, blood samples were collected from 4 randomly selected overnight-fasted rats 3 days post-STZ/NIC by using tail-tip amputation method (Sørensen et al., 2019). Tails of rats were first wiped clean with sterile cotton dipped in 10% ethanol. FBG was measured by using standard glucose peroxidase method (Hall and Keuler, 2009). Rats having consistent FBG (11.1 mmol/L or ≥ 250 mg/dL) over 3 days were considered hyperglycemic (Umamahesh and Veeresham, 2016) and were randomly re-assigned to one of six (6) groups (Fig. 1).

2.13.1. Bodyweight measurements

Bodyweight of rats were measured on day zero and subsequently on days 7, 14, 21 and 28 for rats in all groups.

2.14. Weekly measurement of blood glucose of STZ/nicotinamide-induced diabetic rats

Weekly monitoring of blood glucose concentration was done using a glucometer (Accu-Chek® Active) and glucose test strips. A day before blood glucose measurement, rats were deprived of rodent pelleted chow overnight. Blood samples were collected by tail amputation (Sørensen et al., 2019) early in the morning. FBG was measured on day zero, and days 7, 14, 21 and 28 for rats in all groups.

2.15. Isolation and fixation of pancreas

After general observation of rats in each group, rats were euthanized under chloroform anesthesia. After collection of blood samples from the left ventricle, the pancreas, liver, kidneys, lungs, heart, brain and sections of the small intestines were isolated and preserved in 10% formalin for histological assessments. Also, the left kidney and one liver lobe of representative rats were quickly immersed in normal saline and homogenized for total anti-oxidant capacity (TAC) assessment.

2.16. Blood collection and preparation for anti-oxidant test

The experimental rats used in the *in vivo* studies were sacrificed and blood samples collected via cardiac puncture, dispensed into labeled empty vacutainers. Blood samples were then centrifuged at 3000 rpm (Eppendorf centrifuge 5702, 4 °C) for 5 min to obtain sera.

2.17. Preparation of liver and kidney homogenates for anti-oxidant test

Livers (left lobe) and left kidneys were excised, weighed using an analytical balance, then homogenized in phosphate buffer (pH 7.4) using a homogenizer (WiseTis HG-15D). The homogenates were centrifuged at 4000 rpm at 4 °C for 5 min. The supernatants were collected into labeled Eppendorf tubes and stored at −25 °C prior to use in TAC assessment.

Table 1
Qualitative phytochemical screening on APLE.

Phytochemical Test	Reference	Phyto-compound	Results
Folin Ciocalteu	(Al-Dabbagh et al., 2018; Kupina et al., 2018)	Total phenolic	+
Kumar	Runa et al. (2013)	Flavonoids	+
Salkowski	Abdel-Rahman et al. (2019)	Triterpenoids	–
Keller-Killiani	Dequeker and Loobuyck (1955)	Cardiac glycosides	–
Kumar	Runa et al. (2013)	Anthraquinones	–
Ferric chloride	Moushome et al. (2016)	Tannins	+
Wagner	Moushome et al. (2016)	Alkaloids	+
Froth	Moushome et al. (2016)	Saponins	+

Present (+); Absent (−); APLE – *Abrus precatorius* leaf extract.

2.18. Measurement of total anti-oxidant capacity (TAC) of blood, liver, and kidney

The total anti-oxidant capacity of blood, liver and kidney homogenates was determined by using the phosphomolybdenum method as previously described (Çelik et al., 2014). A reagent solution was prepared containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (0.6 M); mixed in 1:1:1 ratio respectively. A 500 µL of each sample (plasma, liver and kidney homogenates) was in each case mixed with 3 mL of the reagent solution in labeled separate test tubes. Tubes containing reaction solution were incubated at 95 °C for 60–90 min. After cooling to room temperature, absorbance of the reaction solutions was measured at 695 nm using a spectrophotometer against a blank. Ascorbic acid (20–200 µg/mL) was used as standard. Each concentration was prepared in triplicates. A mixture containing 0.3 mL of methanol and 3 mL reagent solution was used as blank. Antioxidant activity was expressed as ascorbic acid equivalent (AscAE).

2.19. Measurement of lipid profile of STZ/NIC-induced diabetic rats

On day 28, after bodyweight measurements and general observations, rats were euthanized under chloral hydrate (100 mg/kg *ip*) anesthesia. Blood samples were collected from rats in the respective treatment groups by cardiac puncture into serum separator tubes. Sera were separated by centrifugation at 2500 rpm for 10 min. Serum cholesterol, serum triglycerides, HDL cholesterol, and LDL cholesterol of rats in respective treatment groups were estimated using a fully automated chemistry analyzer (Mindray BS-2400).

2.20. Histological assessment of pancreas

After isolation of pancreas from anesthetized rats, it was processed for histological assessment according to a previously described method (Feldman and Wolfe, 2014). Briefly, pancreas was fixed in 10% phosphate buffered formalin, processed using an automated tissue processor (Leica TP 1020, Germany) and finally embedded in paraffin wax. Tissue blocks were then cut into sections (at a thickness of 4 µm) by using a rotary microtome. Sections were de-paraffinized and subsequently stained with Hematoxylin & Eosin (H&E). Sections were observed under a microscope (Olympus Model CK40–F200, Japan) by three independent researchers to assess and grade histo-architectural distortions for the various experimental groups. Histomicrographs were generated for representative sections.

2.21. Determination of median cross-sectional area of pancreatic islets

Representative pancreatic sections were scanned to obtain micrographs of all pancreatic islets using an Amscope MD35 eyepiece camera. Each micrograph was uploaded onto Adobe photoshop CS6 and subsequently superimposed by software's stereological grid. The number of intersections overlying the stroma of pancreatic islets was counted. The cross-sectional area of the pancreatic islets of Langerhan's was determined by using Cavalieri method and point counting. The cross-sectional area was measured using the formula:

$$A = \frac{\sum P \times \left(\frac{a}{p}\right)}{M^2}$$

Where:

ΣP - represents the total number of intersections overlying the stroma of pancreatic islets;
a/p - represents the area per point of the stereological grid;
M - Represents the linear magnification.

2.22. Statistical analysis

Data obtained were presented as mean ± SD. Statistical analysis was done by using Graph Pad Prism Version 6 for Windows (Graph Pad Software, San Diego, CA, USA). Mean comparison between groups was done by One - Way ANOVA followed by Tukey's post hoc test. $P \leq 0.05$ was considered statistically significant in all analyses.

3. Results

3.1. Phytochemical, elemental, and proximate profile of APLE

Alkaloids, flavonoids, phenols, saponins, and tannins were detected in APLE (Table 1). Phosphorus, potassium, and sodium were detected in APLE (Table 2). Although calcium, iron and zinc were tested for, none was detected. Proximate composition analysis on APLE showed ash and fibre contents were within standard ranges, while moisture, protein, carbohydrate, and fat contents were below standard ranges (Table 3).

3.2. APLE decreased time-course of blood glucose in normoglycemic rats after oral glucose challenge

Blood glucose kinetics of control rats followed a rise (from 0-30 min), time of glucose peak (TGP, 60 min), and decline (from 90-180 min) reflecting a monophasic glucose response curve. Glucose kinetics in APLE-and-reference (metformin and acarbose)-treated rats were not different from that of control rats except that TGP for APLE was lower (30 min). The shape of the glucose response curve for APLE and reference drugs were monophasic therefore comparable to control (Fig. 2).

3.3. APLE decreased post-prandial blood glucose levels in normoglycemic rats

To assess effect of APLE on post-prandial blood glucose, normal rats were first treated with APLE and reference drugs after overnight fasting, then orally exposed to maltose, lactose and sucrose in separate experiments, then followed by post-prandial blood glucose monitoring

Table 2
Elemental Analysis on *Abrus precatorius* leaf extract (APLE).

Mineral	Amount (µg/100 g)
Phosphorus	0.36 ± 0.02
Potassium	1.88 ± 0.06
Sodium	0.92 ± 0.11
Calcium	ND
Iron	ND
Zinc	ND

ND = not detected; Data are expressed as mean ± standard deviation (SD) of triplicate determinations.

Table 3
Proximate composition of *Abrus precatorius* leaf extract (APLE).

Parameter	Composition (%)
Moisture	10.06 ± 0.12
Ash	9.62 ± 0.30
Protein	10.08 ± 0.56
Carbohydrate	8.09 ± 0.60
Fat	2.48 ± 0.03
Crude fibre	9.37 ± 0.17

Data are expressed as mean ± standard deviation (SD) of triplicate determinations; APLE – *Abrus precatorius* leaf extract; *.

over a two and half hours. Post-prandial blood glucose concentration peaked at 30 min for maltose, lactose and sucrose for all groups (Fig. 3: A1, B1 and C1). However, at all-time points, APLE treatment produced decreased post-prandial blood glucose concentration compared to that of control. Similarly, at all-time points estimated glycated hemoglobin (HbA1c) for APLE-treated rats was lower compared to that of control rats (Fig. 3: A2, B2 and C2).

3.4. APLE decreased elevated blood glucose of STZ/NIC-induced diabetic rats

Exposure of rats to STZ/NIC produced sustained hyperglycemia in rats compared to control rats. However, treatment of STZ/NIC-induced diabetic rats with APLE (100, 200, and 400 mg/kg) dose-dependently decreased elevated blood glucose by 49, 68 and 69% respectively compared to model (25%) (Table 4).

3.5. APLE improved STZ/NIC-induced pancreatic damage in diabetic rats

Pancreatic sections from rats in control group showed normal microstructure of pancreas with high cellularity of the islet of Langerhans compared to those of rats in model group which had vasodilatation of blood vessels and pyknotic nuclei within the acini. Pancreatic sections from STZ/NIC-induced diabetic rats treated with APLE (100 mg/kg) showed reduced vasodilatation of blood vessels, absence of atypical areolar glands in the acini, reduced regeneration of islet cells of Langerhans compared to rats in model group (Fig. 4). Pancreatic sections from rats in APLE (200 mg/kg) group had no ductal necrosis of the exorcise planes present; there was also normal cellularity of the pancreas as a result of regeneration of islet cells of Langerhans compared to rats in model group. Pancreatic sections from rats in APLE (400 mg/kg) group had islet cells arranged as anastomosing cords profused by fenestrating capillaries, and sign of total regeneration of the islet cells of Langerhans compared to rats in model group. Pancreatic sections from rats in metformin group had normal arrangement of islet cells compared to rats in model group (Fig. 4).

3.6. APLE improved median cross sectional area of pancreatic islets of STZ/NIC-induced decrease in pancreatic β-cell mass

The median cross sectional area of the pancreatic islets from control rats was significantly greater ($p < 0.05$) than those of rats in model group. There was a moderate increase in the mean median cross-sectional area of pancreatic islets of APLE treated rats compared to rats in model group, but the difference was not significant ($p > 0.05$) statistically (Table 5). Vasodilatation was common in the model group compared to control group. Also, strips of islet masses in model group were thinner compared to control group and treatment groups.

3.7. APLE prevented bodyweight loss in STZ/NIC-induced diabetic rats

At the end of the 28th day of experimental period, rats in the model group have suffered loss in body weight from day 1 bodyweight by 1.5% compared to control rats which have within the same period gained a mean bodyweight of 34.6%. STZ/NIC-induced diabetic rats treated with APLE (100, 200 and 400 mg/kg) by the end of the 28 days had gained 4.5, 13.1 and 13.2% mean bodyweight respectively compared to model (Table 6). Although there were numerical differences between control and treatment groups with respect to liver/bodyweight ratios, however, the differences were statistically insignificant ($P > 0.05$).

3.8. APLE improved decreased plasma, kidney and liver total anti-oxidant capacity (TAC) of STZ/NIC-induced diabetic rats

Comparatively control rats had increased TAC in plasma, liver and kidney than those rats in the model group. However, treatment of STZ/NIC-induced diabetic rats with APLE (100, 200, and 400 mg/kg po) produced not only increased TAC in plasma, liver and kidney compared to model group but also demonstrated a dose-dependent increase in TAC (Fig. 5). Similarly, metformin treatment of STZ/NIC-induced diabetic rats produced increased TAC in plasma, liver and kidney compared to model group.

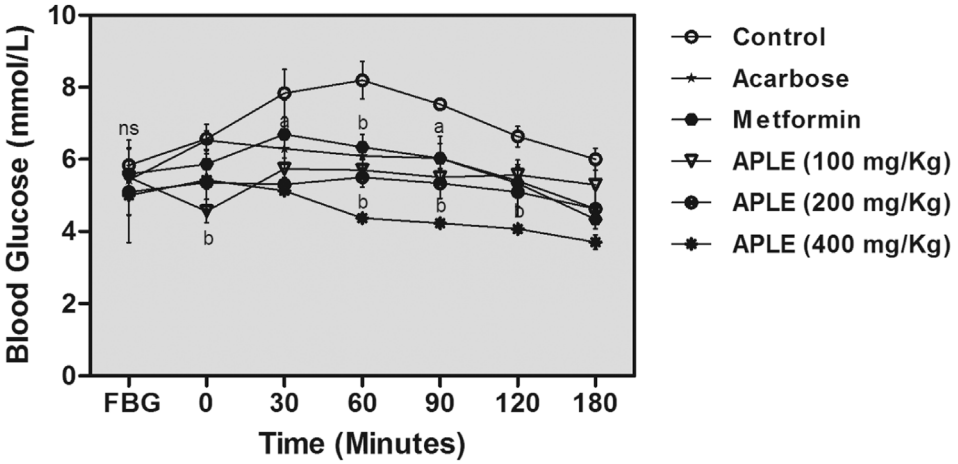

Fig. 2. Effect of APLE and other treatments on blood glucose concentration over a two and half hour period after oral glucose (2 g/kg) challenge. Each point is the mean blood glucose concentration ± SD, n = 3. [a] $P < 0.05$ (Acarbose and metformin vs Control); [b] $P < 0.05$ (APLE vs Control); ns – not significant.

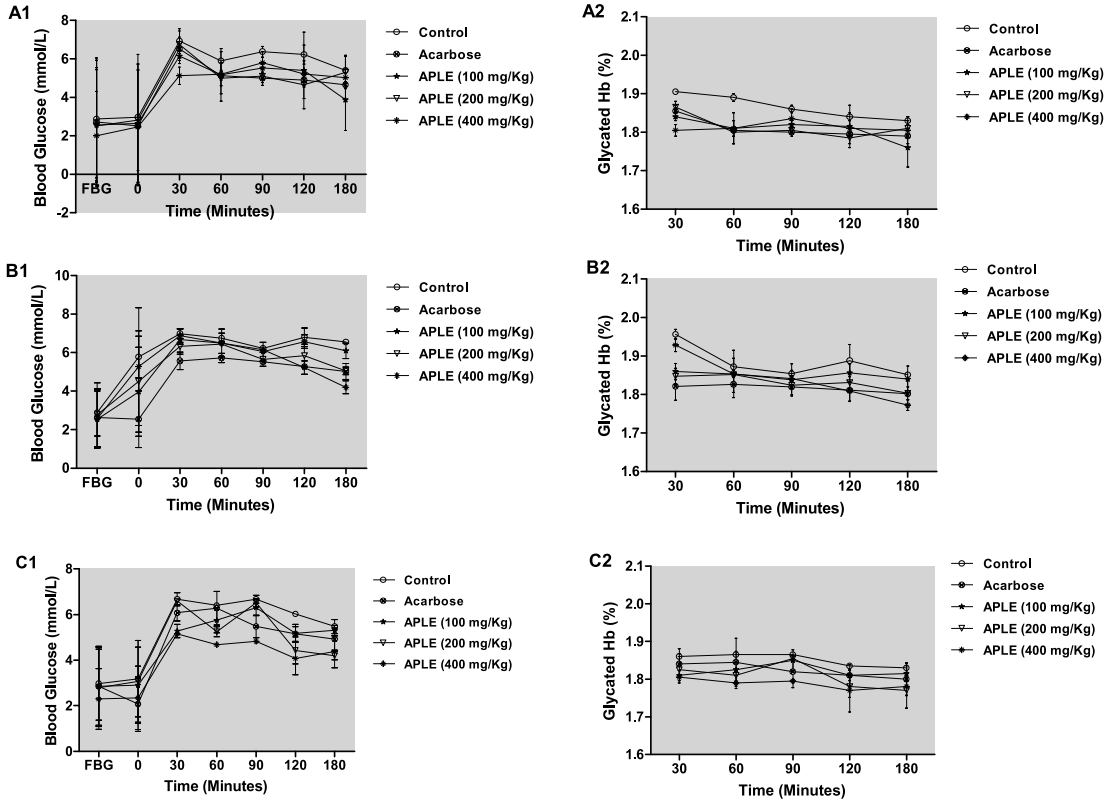

Fig. 3. Effect of APLE and other treatments on post-prandial blood glucose concentration and glycated haemoglobin levels after oral sucrose (A1 and A2), maltose (B1 and B2) and lactose (C1 and C2) challenge. Each point on the curves is the mean blood glucose concentration ± SD, n = 3. APLE – *Abrus precatorius* leaf extract.

3.9. APLE modulates serum lipids of STZ/NIC-induced diabetic rats

Serum levels of triglycerides and cholesterol decreased while HDL increased in control rats relative to STZ/NIC-induced diabetic rats (model). Treatment of STZ/NIC-induced diabetic rats with APLE (100, 200 and 400 mg/kg) decreased serum levels of triglyceride, TC and LDL but slightly increased HDL relative model. TG/TC, TG/HDL and TG/LDL ratios increased in model rats relative to control rats, however, treatment of STZ/NIC-induced diabetic rats succeeded in marginally decreasing these ratios (Table 7).

4. Discussion

Medicinal plants have served mankind in many capacities including general health improvement (Al-Dabbagh et al., 2018), as sources of natural templates for pharmaceutical semi-synthesis in the drug discovery industry (Boye et al., 2020) and even as food. In recent decades, the works of ethnopharmacologists and natural product scientists have advanced knowledge on medicinal plants in the light of their ethnomedicinal uses, bioactive constituents, and ethnopharmacology. Whiles these efforts have succeeded in highlighting the indispensability of herbs and herb-derived products for the existence of mankind (Al-Dabbagh et al., 2018; Amin et al., 2016), on the other hand, it has also revived the unmet need to scientifically verify ethnomedicinal claims on herbs that are central in the primary healthcare of indigenous communities.

The present study demonstrates glucose lowering and pancreatoprotective effects of *Abrus precatorius* leaf extract (APLE) in regulating blood glucose kinetics within normal limits (either monophasic or biphasic glucose response curve with time of peak glucose

Table 4
Cumulative glucose lowering effect of APLE on elevated blood glucose of STZ/NIC - induced diabetic rats.

Treatments	STZ (mmol/L)	Day 28 (mmol/L)	Mean decrease in blood glucose	% Decrease in blood glucose
Control	6.875 ± 0.585	6.125 ± 0.250	0.750 ± 0.335	5.77
*Model	32.500 ± 1.835	19.367 ± 5.229	13.133 ± 3.394	25.32[a]
Met (300 mg/kg)	30.275 ± 9.454	4.725 ± 0.403	25.55 ± 9.051	73.00[b]
APLE (mg/kg)				
100	28.275 ± 6.096	9.650 ± 2.107	18.625 ± 0.989	49.11[b]
200	27.550 ± 10.085	5.075 ± 0.670	22.475 ± 9.415	68.89[b]
400	26.875 ± 10.452	4.850 ± 0.896	22.025 ± 9.556	69.43[b]

Each value is the mean blood glucose concentration ± SD, n = 3.
APLE – *Abrus precatorius* leaf extract.
Met - Metformin.
STZ – Streptozotocin.
* After STZ exposure rats had access to rodent chow and water without treatment.
[a] $P < 0.05$ (Model vs Control).
[b] $P < 0.05$ (APLE or Metformin vs Model).

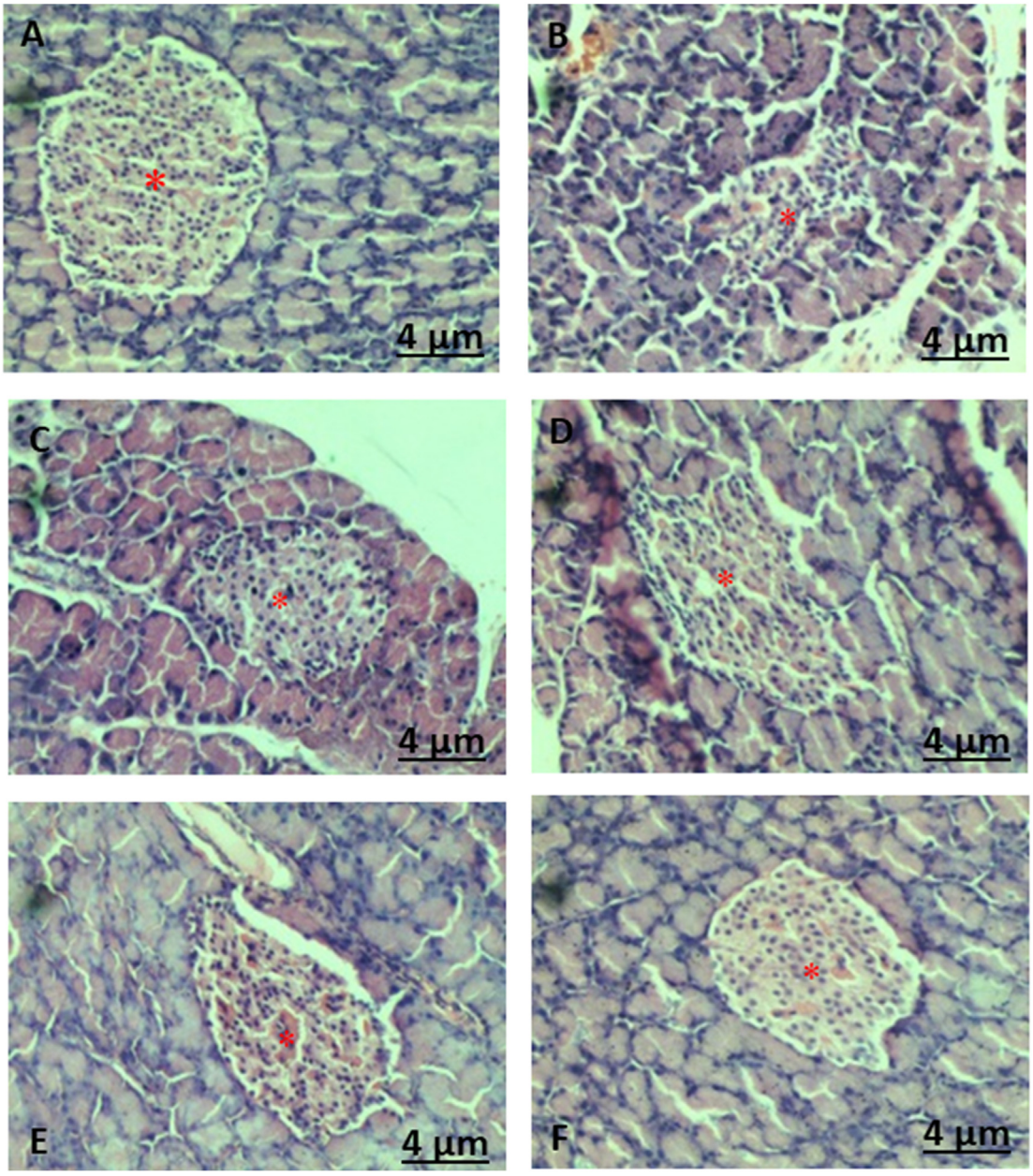

Fig. 4. Effects of APLE and other treatments on pancreatic microstructure of STZ/Nicotinamide - induced diabetic rats. A (Control, x40), B (Model, x40), C (Metformin, 300 mg/kg, x40), D (APLE, 100 mg/kg, x40), E (APLE, 200 mg/kg, x40), F (APLE, 400 mg/kg, x40); APLE – *Abrus precatorius* leaf extract, STZ – Streptozotocin; pink-colored asterisk (*) – pancreatic islet of Langerhans. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

concentration ≥ 30 min) in normoglycemic and STZ/NIC-induced diabetic rats. This initial observation does not only corroborates an earlier report on insulin secretagogue effect of *Abrus precatorius* (Umamahesh and Veeresham, 2016) but also in part confirms the folk use of *Abrus precatorius* leaves in the treatment/management of diabetes mellitus.

Decreased insulin secretion due to defects in pancreatic β-cell function characteristic of type 1 diabetes mellitus and insensitivity of insulin-responsive tissues to insulin characteristic of type 2 diabetes mellitus in each context leads to elevated blood glucose (hyperglycemia). If left untreated over time hyperglycemia may increase the risk of micro-vascular complications such as nephropathy, retinopathy, and neuropathy just to mention but a sample. Of note, impaired glucose tolerance (IGT) and Impaired fasting glucose (IFG) are two different clinical manifestations of impaired glucose metabolism (Morita et al., 2006) and these are routinely used to assess risk of pre-diabetes or as a means to assess glycemic control among diabetics. Also, morphologic characteristics of blood glucose response-curves (fasting blood glucose [FBG], shape of curve, either monophasic or biphasic, time to glucose peak [TGP], and 2-hour blood glucose concentration) from 2-hour oral glucose tolerance test (OGTT) reflect physiologically different phenotypes of insulin secretion and action (Kim et al., 2016). For example, TGP could predict risk of pre-diabetes and also used to predict pre-diabetes risk stratification (Chung et al., 2017). A TGP ≥30 min is associated with increased odds of pre-diabetes in human adults without diabetes (Chung et al., 2017). In the present study orally challenging normoglycemic rats with glucose after APLE treatment produced a characteristic glucose response-curve that reflected normal kinetics of glucose including FBG (below 6 mmol/L), TGP (30 min) and a 2 h blood glucose concentration (4.1–5.8 mmol/L) relative to control which had

Table 5
Effect of APLE on median cross sectional area of pancreatic islets of Langerhans of STZ/nicotinamide-induced diabetic rats.

Treatment Groups	N	Area of pancreatic islets	I.Q. range (x 10^6 μm^2)	K	p-value
Control	4 (50)	87.21 × 10^6 μm^2	25.30–163.10	25.38	0.0001
*Model	3 (117)	23.47 × 10^6 μm^2 [a]	18.78–42.25		
Met (300 mg/k)	4 (88)	37.56 × 10^6 μm^2 [b]	14.08–86.65		
APLE (mg/kg)					
100	4 (31)	32.86 × 10^6 μm^2 [b]	12.91–51.64		
200	4 (42)	28.17 × 10^6 μm^2 [b]	18.78–75.11		
400	4 (100)	32.86 × 10^6 μm^2 [b]	14.08–65.72		

Each value is the mean blood glucose concentration ± SD, n = 3. N = number of pancreas examined. () = total number of pancreatic islets from pancreas of representative rats in each group.
APLE – *Abrus precatorius* leaf extract.
Met – Metformin.
STZ – Streptozotocin.
I.Q. represents interquartile range.
K represents Kruskal Walli's test statistic.
* STZ/Nicotinamide – induced diabetic rats had no treatment except access to rodent chow and water.
[a] P < 0.05 (Model vs Control).
[b] P < 0.05 (APLE or Metformin vs Model).

much higher values under the same condition (Fig. 2). Also, oral exposure of disaccharides (maltose, lactose, and sucrose) to normoglycemic rats post-APLE treatment produced a characteristic blood glucose response-curve (Fig. 3: A1, B1 and C1) similar to the earlier observation (Fig. 2) and corroborated by a potent and sustained glucose lowering effect of APLE on elevated blood glucose in rats over a 28 day period induced by STZ/NIC (Table 4). Clearly, APLE has consistently demonstrated glucose lowering effect in these models of diabetes mellitus but the mechanism remains unknown. However, utilization of glucose by cells is in part limited by rate of absorption (uptake of glucose into cells) by cells secondary to insulin secretion and action. STZ is one of the main diabetogenic agents that exhibit specific toxicity to pancreatic β-cells. STZ enters pancreatic β-cells through glues transporter (GLUT2) and cause DNA alkylation. DNA alkylation induces activation of ADP-ribosylation, a process indispensable for the diabetogenic effect of STZ. Subsequently, depletion of NAD^+ and ATP secondary to poly-ribosylation in combination with donation of nitric oxide (NO) lead to generation of unstable chemical molecules including superoxide radicals which have the potential to cause damage to pancreatic β-cells. These cellular events decrease pancreatic β-cell mass via necrosis followed by decreased insulin secretion and action as well as a hyperglycemia episode. In the light of this, recovery of damaged pancreatic β-cells just as pancreato-protection is indispensable for effective treatment of diabetes. From the present study, APLE recovered damaged pancreatic β-cells in a dose-dependent manner after 7 days exposure of rats to STZ/NIC and also by not-too clear mechanism increased glucose uptake inferring from the significant recovery of pancreatic β-cells and increase in pancreatic β-cell mass (Fig. 4 and Table 5). Pancreatic β-cell mass directly relates to insulin secretion and under normal condition insulin action, therefore APLE treatment-related increase in pancreatic β-cell mass perhaps related directly to increase in insulin secretion and action that resulted in increased glucose uptake and decrease in blood glucose.

Hyperglycaemia is associated with increased catabolism. Retention of glucose in blood due to inability of cells to take up glucose occasions a hyperosmolar blood that may also lead to increase in blood volume and attendant vascular disorders. Also, cells are deprived of cellular fuel (glucose) which compels body cells to resort to alternative energy sources including body fats and proteins. These series of cellular events leads to dysregulated cellular metabolism. The balance between catabolism and anabolism becomes disrupted with increased catabolism (extraction of cellular energy from body fats and proteins instead of glucose) at the expense of anabolism which occasions chronic loss in bodyweight. From the present study, STZ/NIC-induced diabetic rats (model group) suffered chronic loss in bodyweight while APLE-treated rats either sustained their bodyweight or gained weight (Table 6), indicating that APLE treatment restored the balance between catabolism and anabolism in the STZ/NIC-induced diabetic rats secondary to recovery of damaged pancreatic β-cells, and this observation mirrors an earlier report on the anti-hyperglycaemic effect of *Costus pictus* leaf extract in rats (Gireesh et al., 2009).

Glycated haemoglobin (HbA1c) is one of the minor hemoglobins (HbA_{1a1}, HbA_{1a2}, and HbA_{1b}), normally identified by using cation exchange chromatography. HbA1c is produced throughout the life cycle of a red blood cell via a non-enzymatic post-translation modification of haemoglobin molecule. During this structural post-translational modification, glucose binds to the N-terminal valine residue of β-chain of haemoglobin via a two-stage reaction. First, the aldehyde moiety of glucose partakes in a reaction to form a Schiff base which results in the formation of a labile aldimine. Normally, most of the labile aldimine dissociates to form HbA0 and free glucose, however, a small fraction of the labile aldimine undergoes irreversible Amadori re-arrangements to form a stable ketoamine (HbA1c). The concentration of the resultant HbA1c is ten-fold higher than all the other minor haemoglobins (HbA_{1a1}, HbA_{1a2}, and HbA_{1b}) put together. While the concentration of all the other minor haemoglobins (HbA_{1a1}, HbA_{1a2}, and HbA_{1b}) does not fluctuate to reflect the progression of diabetes mellitus, the concentration of HbA1c fluctuates to reflect progression of diabetes

Table 6
Effect of APLE on Bodyweight and Liver weight/bodyweight Ratios of STZ/nicotinamide-induced Diabetic Rats.

Treatment Groups	Mean Bodyweight (g)m		Change in mean body weight	% change in mean body weight	Liver weight (g)	[a]Liver weight/mean body weight ratio
	Day 1	Day 28				
Control	137.25 ± 7.49	184.8 ± 11.3	47.55 ± 3.81	34.644	6.94 ± 1.40	0.038 ± 0.124
*Model	133.00 ± 8.96	131.00 ± 8.62	−2 ± 0.34	1.50[a]	6.19 ± 0.32	0.047 ± 0.037
Met (300 mg/kg)	130.75 ± 6.25	158.75 ± 7.48	28 ± 1.23	21.41[b]	6.32 ± 1.01	0.040 ± 0.135
APLE (mg/kg)						
100	121.8 ± 10.2	127.25 ± 16.9	5.45 ± 6.7	4.47	6.18 ± 1.28	0.049 ± 0.076
200	127.25 ± 8.27	144.25 ± 8.26	17.0 ± 0.01	13.36[b]	6.32 ± 1.03	0.044 ± 0.125
400	134.75 ± 8.04	152.5 ± 10.6	17.75 ± 2.56	13.17[b]	6.58 ± 1.25	0.043 ± 0.118

Each value is the mean blood glucose concentration ± SD, n = 3.
APLE – *Abrus precatorius* leaf extract.
Met – Metformin.
STZ – Streptozotocin.
* After STZ exposure rats had access to rodent chow and water without treatment.
[a] P < 0.05 (Model vs Control).
[b] P < 0.05 (APLE or Metformin vs Model).

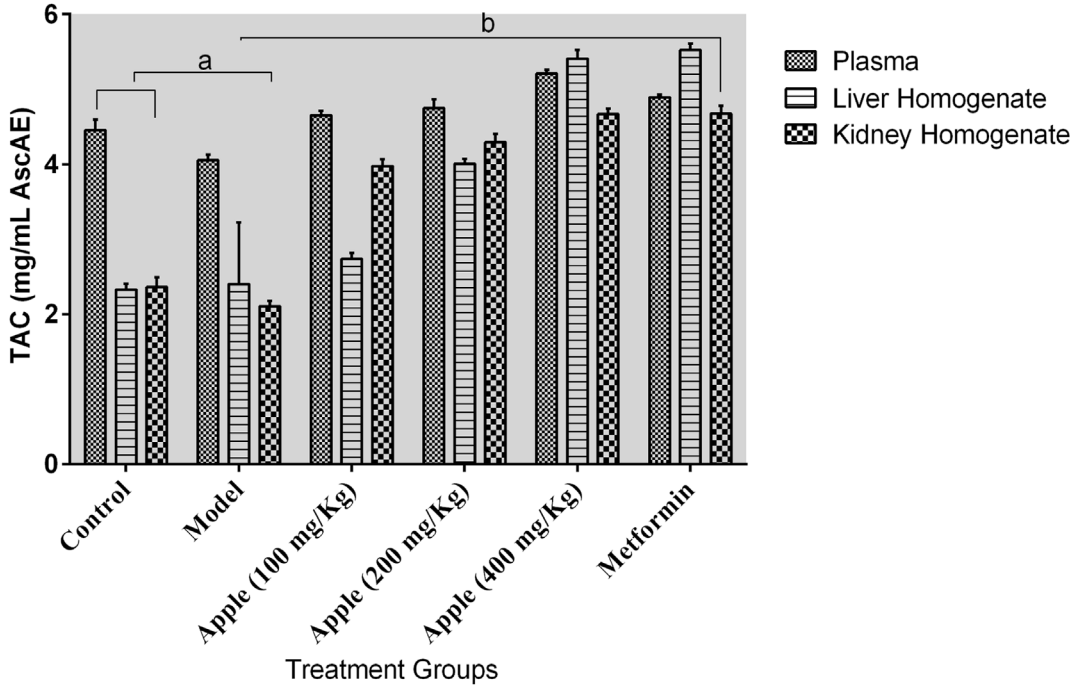

Fig. 5. Effect of APLE on total anti-oxidant capacity (TAC) of STZ/Nicotinamide-induced diabetic rats. Each bar is the mean ± SD, n = 3. [a] P < 0.05 (Model vs Control); [b] P < 0.05 (APLE and Metformin vs Model group).

mellitus, specifically HbA1c increases in diabetics. Therefore, HbA1c is predictive of progression of diabetes mellitus as well as glycaemic management/control. In the present study rats treated with APLE before oral challenge with various disaccharides (maltose, lactose and sucrose) did not only effectively decrease postprandial blood glucose over a 2-h period comparable to control but also decreased HbA1c (Fig. 3: A2, B2 and C2). Secretion and activity of hydrolytic enzymes such as α-glycosidase represent a rate-limiting factor for hydrolysis of most disaccharides (maltose, lactose and sucrose) into monosaccharaides (mainly glucose), therefore APLE may have inhibited the secretion and activity of these hydrolytic enzymes leading to slowed release of glucose from disaccharides. Also, increased senescence of aged red blood cells but increased population of young erythrocytes (increased erythropoiesis) favours decreased concentration of HbA1c in view of the fact that HbA1c is produced throughout the life cycle of a red blood cell. Aged red blood cells therefore contain more HbA1c than young red blood cells. It is possible APLE promoted erythropoiesis while increasing apoptosis of aged red blood cells which additively led to decrease in HbA1c concentration.

In a hyperglycemic episode, intracellular hyperglycemia results in generation and shunting of more electron donors such as NADH and $FADH_2$ into the mitochondrial electron transport chain. Voltage across the membrane above threshold results in inhibition of protein complex III, buildup of electrons in coenzyme Q and subsequent delivery of more electrons to oxygen to form superoxide. Buildup of excess superoxide in pancreatic β-cells results in depletion of anti-oxidants including superoxide dismutase. Inability of resident anti-oxidants to mop up reactive oxygen species (ROS) including superoxide results in DNA

Table 7
Effect of APLE on serum lipid levels of STZ/Nicotinamide-induced Diabetic Rats.

Treatment Groups	(mg/dL)				TG:TC	TG:HDL	TG:LDL
	TC	HDL	TG	LDL			
Control	1.30 ± 0.21	1.64 ± 0.20	0.49 ± 0.17	0.64 ± 0.08	0.367	0.299	0.77
Model*	1.14 ± 0.03	1.53 ± 0.03	1.15 ± 0.55	0.36 ± 0.08[a]	1.01	0.75	3.19
Met (300 mg/kg)	0.89 ± 0.42[b]	1.14 ± 0.47	0.51 ± 0.26[b]	0.34 ± 0.23[b]	0.57	0.45	1.5
APLE (mg/kg)							
100	1.09 ± 0.18	1.34 ± 0.28	0.37 ± 0.09[b]	0.64 ± 0.03	0.34	0.28	0.58
200	0.98 ± 0.20[b]	1.06 ± 0.50	0.46 ± 0.21[b]	0.59 ± 0.31[b]	0.47	0.43	0.78
400	0.92 ± 0.45[b]	1.22 ± 0.50	0.56 ± 0.29[b]	0.22 ± 0.16[b]	0.61	0.46	2.55

Each value represents mean ± SD, n = 3.
APLE – *Abrus precatorius* leaf extract.
Met – Metformin.
STZ – Streptozotocin.
HDL – High Density Lipoprotein.
LDL – Low Density Lipoprotein.
TC – Total Cholesterol.
TG – Triglyceride.
* After STZ/Nicotinamide exposure rats had access to rodent chow and water only without treatment.
[a] P < 0.05 (Model vs Control).
[b] P < 0.05 (APLE or Metformin vs Model).

depletion of anti-oxidants such as superoxide dismutase, increase in DNA damage and eventual cell death. From the present study, APLE treatment increased total anti-oxidant capacity in blood, kidney and liver relative to model rats (Fig. 5), indicating that APLE treatment indeed re-activated resident anti-oxidants and also perhaps increased free radical scavenging.

Insulin resistance (IR) is a key phenotypic hallmark of type 2 diabetes mellitus and it is associated with dyslipidemia (Zhou et al., 2016). In support, an association has been reported between hypercholesterolemia and type 2 diabetes mellitus (Besseling et al., 2015), while triglyceride/HDL-C ratio is also reported as a strong predictor of insulin resistance but not pancreatic β-cell function (Zhou et al., 2016). From the present study, not only was triglycerides and total cholesterol increased in the STZ/NIC-induced diabetic rats but also all the triglyceride-based ratios confirmed at least in the rats used in this study that indeed dyslipidemia is associated with IR as previously reported (Zhou et al., 2016). APLE treatment of STZ/NIC-induced diabetic rats over 28 days restored lipid metabolism decreasing triglyceride and total cholesterol levels (Table 7).

Diabetics as part of non-pharmacological therapy are advised to eat foods having low glycaemic index (Ojo et al., 2018; Thomas and Elliott, 2009) and also avoid foods that may disrupt electrolyte balance (de Carvalho et al., 2017; Shirzaiy et al., 2015). Phosphorus, potassium and sodium but not calcium, zinc and iron were detected in APLE (Table 2), and all the detected elements were at concentrations lower than the minimum limit of the daily intake required normally from foods, indicating that exposure of APLE to humans will not contribute undue additional exogenous electrolytes to induce electrolyte imbalance. Also, from the proximate composition assessments, small percentage of carbohydrate was detected relative to protein in APLE (Table 3); perhaps explaining in part why APLE could restore loss in bodyweight in STZ/NIC-induced diabetic rats.

Preliminarily, an association has been reported between hypercholesterolemia and type 2 diabetes mellitus (Besseling et al., 2015). From the present study, APLE treated rats had decreased levels of lipids relative to STZ/NIC-induced diabetic rats (Table 7). As much as these lipids, particularly cholesterol and triglycerides are associated with type 2 diabetes mellitus, the present observation could be directly attributed to saponins which were among the phytocompounds detected in APLE. Saponins form insoluble complexes with 3-hydroxysteroids leading to formation of large micelles with bile acids and cholesterol (Vinarova et al., 2015) therefore interfere with both biosynthesis and absorption of cholesterol and its precursors. The phytocompounds detected in APLE, particularly saponins may play key role in reducing the levels of cholesterol and triglycerides as confirmed by earlier studies (Vinarova et al., 2015).

Herbs used in herbal medicine therapy owe their medicinal, biological, and pharmacological properties to their constituent secondary plant metabolites. Of note, flavonoids, saponins, tannins and alkaloids are not only common in most medicinal plants but also have demonstrated glucose lowering effect in animal models of diabetes mellitus (Alam et al., 2019; Ponnusamy et al., 2011; Tafesse et al., 2017; Zhang et al., 2018). Phenolic compounds, mostly tannins and flavonoids exert blood glucose lowering effect and this was demonstrated experimentally in rats (Kumkrai et al., 2015). Tannins are shown to complex with mainly proteins and other macromolecules to modulate their pharmacokinetics, specifically solubility and absorption. Similarly, saponins have demonstrated hypocholesterolemic effect by forming complexes with cholesterol and its precursors (bile salts) thereby decreasing their absorption and biosynthesis. From the present study, phenols, flavonoids, tannins, saponins and alkaloids were identified in APLE and this observation is consistent with previous reports on *Abrus precatorius* (Garaniya and Bapodra, 2014). Importantly, the glucose lowering effect demonstrated by APLE treatment is attributable to its phytocompounds (Table 1) in view of the earlier reports which show that all the phytocompounds detected in APLE by varied mechanisms possess glucose lowering effects (Kumkrai et al., 2015).

5. Conclusion

APLE has demonstrated in rats glucose lowering and pancreatoprotective effects and has also demonstrated the ability to restore loss in bodyweight associated with diabetes mellitus, confirming its folk use as an anti-diabetic herbal medicine.

Acknowledgement

We specially acknowledge the traditional healers from Sefwi Asawinso Kitiwamu in the western region of Ghana for volunteering information on the traditional uses of *Abrus precatorius* in their community.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112918.

Abbreviations

APLE	*Abrus precatorius* leaf extract
AscAE	Ascorbic acid equivalent
AOAC	Association of Official Analytical Chemists
DM	diabetes mellitus
IR	insulin resistance
NIH	National Institute of Health
ROS	reactive oxygen species
STZ	streptozotocin
TAC	total anti-oxidant capacity

Author contribution

AB: conceived, designed and wrote the manuscript.

DOA: Read and edited the final manuscript together with AB for intellectual content.

JKA: Performed all chemistry-related experiments.

DAM, ASB and PJA: Performed experiments and literature search.

EOG and EAA: performed experiments and analyzed data.

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