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Original research

Ganoderma lucidum ethanol extract abrogates metabolic syndrome in rats: *In vivo* evaluation of hypoglycemic, hypolipidemic, hypotensive and antioxidant properties

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ABSTRACT

Background: Metabolic syndrome (MetS), characterized by hyperglycemia, dyslipidemia, hypertension, and central obesity remains a global menace. Ganoderma lucidum possesses various beneficial bioactivities. This study, thus, evaluated the efficacy of Ganoderma lucidum ethanol extract (GLEE) against MetS complications in rats.

Methodology: Thirty male rats were randomized into six groups (n = 5): Control, MetS control, MetS + standard drugs, MetS + GLEE (26 mg/kg), MetS + GLEE (44 mg/kg), and MetS + GLEE (70 mg/kg). Bodyweight, blood sugar, and pressure were monitored. Animals were sacrificed following two weeks of GLEE treatment post-MetS induction. Blood, pancreas, heart, liver, and kidney were collected for biochemical and histopathological analyses. Plasma triacylglycerol (TAG), plasma and lipoproteins (HDL and LDL) cholesterol (CHOL) levels, and activities of superoxide dismutase (SOD), and catalase (CAT), as well as malondialdehyde (MDA) level, were estimated in the pancreas, heart, liver, and kidneys.

Results: GLEE Phyto-analysis revealed terpenoids, flavonoids, alkaloids, and saponins. A dose-dependent total antioxidant capacity and, a near dose-dependent DPPH radical scavenging, and ferric ion reducing ability were exhibited by GLEE in vitro. GLEE (70 mg/kg) reversed significantly ($p < 0.05$) the MetS-induced hyperglycemia and hypertension. Furthermore, increments in plasma TAG, CHOL, LDL, and MDA levels reduced dose-dependently. Increased CAT (pancreas and heart) and SOD (the four organs) activities and, NRF2 protein levels significantly reduced in GLEE-treated group relative to MetS control. Histological evidence suggests that GLEE abated the MetS- induced cytomorphological derangements in the organs.

Conclusion: This study concludes that GLEE may be a viable regimen against MetS and its attendant complications.

1. Introduction

Metabolic syndrome (MetS), characterized by hyperglycemia, dyslipidemia, hypertension, and central obesity is a global public health menace (Nilendra and Scmhrd, 2018). These metabolic diseases are the primary risk factors for the development of cardiovascular diseases (CVDs) that prelude type II diabetes (T2D), and other micro-and macrovascular disorders (Nilendra and Scmhrd, 2018). World Health Organisation (WHO) and other related agencies define MetS under five cardinal points namely: abdominal obesity/excessive waist circumference, hypertension, excessively high fasting blood sugar, hypertriglyceridemia, and low level of high-density cholesterol (HDL) (NCEP, 2002; Ansari-Moghaddam et al., 2019). MetS is a widespread and global health concern that has attracted much attention due to economic burden and increased morbidity and mortality rate (Zhou et al., 2014; Kelli et al., 2015). An estimated 18 million mortalities were recorded worldwide due to CVDs. Alarmingly, the majority of the reported deaths occurring in the developing and low-income countries (Kelli et al.,

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2015). Alarmingly, predictions have it, that a quarter of the world's adult population has metabolic syndrome stemming from multiplex predisposing factors such as race, age, gender, nutritional habit, genetic, and epigenetic differences (Damiri et al., 2018). Indeed, MetS in children is even difficult to diagnose due to a continuous upsurge in the number of childhood obesity and inability to measure some of the cardinal signs of MetS accurately in them making prevention from childhood a herculean task (Damiri et al., 2018). Obesity prevalence continues to rise yearly, thereby, deepening the growing cases of MetS and its complications on a global scale (Kelli et al., 2015). To flatten the ever-rising curve of MetS cases, preventive and therapeutic strategies must focus on abating the clinical manifestations of MetS such as attenuating visceral obesity, diabetic Mellitus, hypertension, insulin resistance, and dyslipidemia (Grundy et al., 2005; Kelli et al., 2015). The chemotherapeutic approach has helped in the management of MetS complications. However, high cost, systemic adverse side effects of these allopathic drugs, and the inability of a single drug to abolish all the clinical manifestations of MetS warrants a continuous screening for cheap and readily available natural products (Rajarathnam et al., 2003).

Mushrooms have gained scientific scrutiny in the treatment and management of myriads of pathologies worldwide. Drug formulations produced from mushrooms include tablets, capsules, and extracts (Rajarathnam et al., 2003). Ganoderma lucidum (Curtis) P Karst, belongs to the genus of polypore fungi of the family Ganodermataceae that includes about 80 species found in many from tropical regions (Kirk et al., 2010). They are white-rot fungi with enzymes that allow them to break down wood components, such as lignin and cellulose (Loyd et al., 2018). Ganoderma lucidum (Curtis) P. Karst embodies various metabolites such as polysaccharides and triterpenes, from which it derived its pharmacologic effects. Indeed, Zhang and Lin (2003), and Berger et al. (2004) suggested that G. lucidum was well tolerated by the human body, and might improve multiple cardiovascular risk factors including hypertension, hyperglycemia, and dyslipidemia. Oxidative stress arising from an imbalance between the production and elimination of reactive oxygen species (ROS) and disruption of the antioxidant systems are implicated in the development and progression of insulin-resistant (IR) (Chartoumpekis and Kensler, 2013). Therefore, modulation of ROS-producing or eliminating molecular pathways is a potential target for MetS treatment. In this regard, we pose that NF-E2-related factor 2 (Nrf2) - an antioxidant responsive transcription factor might be a molecular target in the management of MetS (Kensler et al., 2007). Furthermore, despite the reported biomedical benefits of G. lucidum, enough empirical data regarding its safety, efficacy, and potency in the management of MetS and its complications remains enigmatic (Hapuarachchi et al., 2016). This study thus investigated the potential abrogative effect of Ganoderma lucidum ethanol extract (GLEE) on metabolic syndrome induced in rats by evaluating its anti-dyslipidemic, anti-hypertensive, anti-hyperglycemic, and antioxidant effects in rat tissues.

2. Materials and methods

2.1. Collection of materials and ethical approval

Ganoderma lucidum (Curtis) P. Karst Fruiting bodies were collected from the Botanical gardens, University of Ibadan, Oyo State, Nigeria, and identified by Professor Clementina O. Adenipekun of the Department of Botany, University of Ibadan. Afterwards, it was washed, oven-dried at 45 °C, ground into powdered form, and kept in a desiccator pending use. Ethical approval was obtained, and the experimental protocols conform to ethical guidelines of Animal Care and Use of Research Ethics Committee (UI-ACUREC), University of Ibadan, Oyo State, Nigeria.

Table 1
Composition of experimental diets used in the study.

Constituents	Standard Diet	High Fat Diet
Crude Protein	11.0%	11.0%
Crude Fat	4.0%	4.0%
Crude Fibre	5.0%	5.0%
Calcium	1.0%	1.0%
Phosphorus	0.47%	0.47%
L-lysine	1.15%	1.15%
Methionine	0.5%	0.5%
Metabolic energy (calorie)	2900	2900
Lard		20%

2.2. Preparation of G. lucidum ethanol extracts (GLEE)

Powdered G. lucidum (2 g, 5 g, and 10 g) was boiled in of200 mL of absolute ethanol separately for 10 min; the resulting solution was sieved and drained to remove the shaft. The shaft was oven-dried, and the dry weight recorded. The decocted extract was used for treatment.

The formula; [(wet weight-dry weight)/Vol of ethanol used] was used, to calculate the concentration of Ganoderma lucidum extracted in the resulting solution. We obtained the concentration of 3.25 mg/mL, 5.5 mg/mL, and 8.75 mg/mL from 2 g, 5 g, and 10 g of the powdered G. lucidum respectively. The concentrations are equivalent to 6.5-, 11-, and 44 mg/kg body weight in that order, as per the American Herbal Pharmacopoeia guideline (2006). Thus, 26 mg/kg, 44 mg/kg, and 70 mg/kg doses respectively were administered to the animals at 2 mL/kg body weight.

2.3. Qualitative and quantitative screening of G. lucidum

Qualitative evaluation of terpenoids, steroids, saponins, flavonoids, tannins, cardiac glycosides, alkaloids, phenols, and anthraquinones contents of G. lucidum were as per the methods described by Ayoola et al. (2008). Quantitative measurement of alkaloids, flavonoids, and saponins content was done according to the method of Santhosh et al. (2016). Saponins level was quantified as described by Brunner (1984), while the terpenoid content was estimated according to the method of Indumathi et al. (2014).

2.4. Proximate analysis of GLEE

The estimate of the various food parameters namely: moisture contents, total ash, crude fat, crude protein, total carbohydrate on the ground powder of the G. lucidum mushroom as well as the feed was done according to the method of Association of Official Analytical Chemists (A.O.A.C., 2010).

2.5. In vitro antioxidant profiling of GLEE

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the G. lucidum was evaluated as described by Manzocco (1998) and ferric ion reducing antioxidant potential (FRAP), and total antioxidant capacity (TAC) assays as described by Oyaizu (1986). Ascorbic acid was used as the positive reference standard. All assays were done in triplicate.

2.6. Composition of high fat diet (HFD) and standard experimental diets

HFD used was composed of 20% lard together with other components as described by Shi et al. (2011) as shown in Table 1.

2.7. Experimental animals, induction, and design

2.7.1. Experimental animals

Male Wister rats (30) weighing between 120 and 130 g obtained

Table 2
Experimental design and substance administered.

Groups	Administration
Control	Standard diet + clean water
Metabolic syndrome (MetS)	HFD + 10% fructose + STZ
MetS + Standard Drugs	MetS + 6 mg and 25 mg/kg BW Glibenclamide and Atenolol
MetS + GLEE (26 mg/kg)	MetS + GLEE (26 mg/kg BW)
MetS + GLEE (44 mg/kg)	MetS + GLEE (44 mg/kg BW)
MetS + GLEE (70 mg/kg)	MetS + GLEE (70 mg/kg BW)

BW- body weight; MetS- metabolic syndrome; GLEE- *Ganoderma lucidum ethanol extracts, STZ*- Streptozotocin, HFD- High Fat Diet.

were from the Animal House Unit of the Department of Physiology, College of Medicine, University of Ibadan, Oyo State, Nigeria. All rats were maintained under hygienic conditions and handled humanely. After acclimatization to laboratory conditions for two weeks, all rats had access to high-fat diet (HFD) and water except the Normal control group that received standard diet.

2.7.2. Induction of metabolic syndrome (MetS) in rats

We induced an experimental model of MetS in rats by single intraperitoneal administration of freshly prepared streptozotocin (STZ) (50 mg/kg in 0.1 M citrate buffer, pH 4.5) combined with HFD and 10% fructose. The combined effect of HFD, fructose solution, and STZ injection will presumably induce a Type – 2 diabetes (T2D), hyperglycemia, dyslipidemia, and hypertension in the rats (Srinivasan and Ramarao, 2007; Mamikutty et al., 2014). Briefly, the animals were maintained on HFD and 10% fructose/or standard diet (Normal control) until they attained a weight range between 250 and 290 g before the injection of the STZ. After 72 h of STZ administration, rats with fasting blood glucose >200 mg/dL were considered diabetic. A non-invasive automatic curve inflation method used to estimate the rat blood pressure was as per Meidert and Saugel (2018). Rats with systolic pressure of over 140 and diastolic of 100, were considered hypertensive and used for the study. Bodyweight monitoring was throughout the experimental duration.

2.7.3. Experimental design

After the confirmation of metabolic syndrome (MetS) in the rats, 30 rats (containing 25 MetS and 5 normal rats) were randomly divided into six groups (n = 5) as follows: (See Table 2)

2.8. Animal sacrifice and tissue collection

After two weeks of treatment, animals euthanized followed an overnight fast. The animals were handled humanely according to the guides on experimental animals use as described by Rowett (1977). Blood was collected via the retro-orbital plexus into anticoagulant-containing tubes. Rats were sacrificed by cervical dislocation after which the pancreas, heart, liver, and kidneys were excised and stored in a freezer for further analysis.

2.9. Blood chemistry

2.9.1. Fasting blood glucose determination

Blood glucose was determined using a digital glucometer (Accu-check ® sensor, Roche Diagnostics GmbH Mannheim, Germany).

2.9.2. Lipids profile analysis

Plasma triacylglycerol (TAG) and cholesterol (CHOL) concentrations were measured spectrophotometrically, using Randox test kits (Randox Laboratories, England), as directed by the manufacturers' manuals. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) -cholesterol was calculated using Friedwald's method (Friedwald et al., 1972).

Table 3
Qualitative and Quantitative analyses of phytochemicals present in *Ganoderma lucidum ethanol extracts* (GLEE). Data are expressed as mean ± SEM (n = 3). + - present.

Phytochemical	Alkaloids	Flavonoids	Saponins	Terpenoids
Qualitative screening	+	+	+	++
Quantitative screening (%)	12 ± 2.28	17 ± 0.00	0.75 ± 0.71	25 ± 1.41

++ - abundant.

Table 4
Nutritional proximate analyses of *GLEE*.

Moisture	Crude fibre	Ash	Fat	Crude protein	Carbohydrate
17.84 ± 0.00	8.16 ± 0.30	8.5 ± 0.50	0.03 ± 0.00	11.79 ± 0.2	53.68 ± 0.18

Values are expressed as mean ± standard error of mean (SEM). Analyses was done in triplicate (n = 3). All values are expressed as percentage weight/weight (%w/w).

2.9.3. In vivo oxidative stress and enzymatic antioxidant determination

Malondialdehyde (MDA) level estimated was as described Beuge and Aust (1978). Assay for superoxide dismutase (SOD) and catalase (CAT) activity were according to the methods of Marklund and Marklund (1974), Mahmood, and Abed (2016), respectively.

2.9.4. Quantification of NRF2 levels in the pancreas, heart, liver, and kidney

Quantitative measurement of the level of NRF2 level was by Mini Enzyme-Linked Immunosorbent Assay (ELISA) Development Kits (Peprotech). A total of 96-well plates were set up according to the manufacturer's instructions and read using an ELISA plate reader at 405 nm with 650 nm as the correction wavelength. Concentrations of the NRF2, was estimated in the tissue homogenates.

2.9.5. Tissues histological evaluation

A section of the liver, kidney, heart, and pancreas was cut and preserved in 10% formalin for histo-morphological evaluation.

2.9.6. Statistical analysis

All data was represented as the mean ± standard error of mean (SEM). To evaluate the differences among means between the test and control group, data was analysed by a one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS), version 16.0. P-value < 0.05 was considered significant. Duncan multiple range test was used to determine the significance level among groups. Graphs were plotted using GraphPad Prism Software (Version 5).

3. Results

3.1. Phytochemical constituents of Ganoderma lucidum ethanol extract (GLEE)

The qualitative and quantitative phytochemical composition of *Ganoderma lucidum* are presented in Table 3.

Four prominent phytochemical constituents were detected in the GLEE namely: saponins, flavonoids, terpenoids, and alkaloids with terpenoids being the most abundant. Quantitative analysis revealed that ethanol extract of *Ganoderma lucidum* had Terpenoids (25%), Flavonoids (17%), and Alkaloids (12%) while Saponins (0.75%) was the least quantity detected.

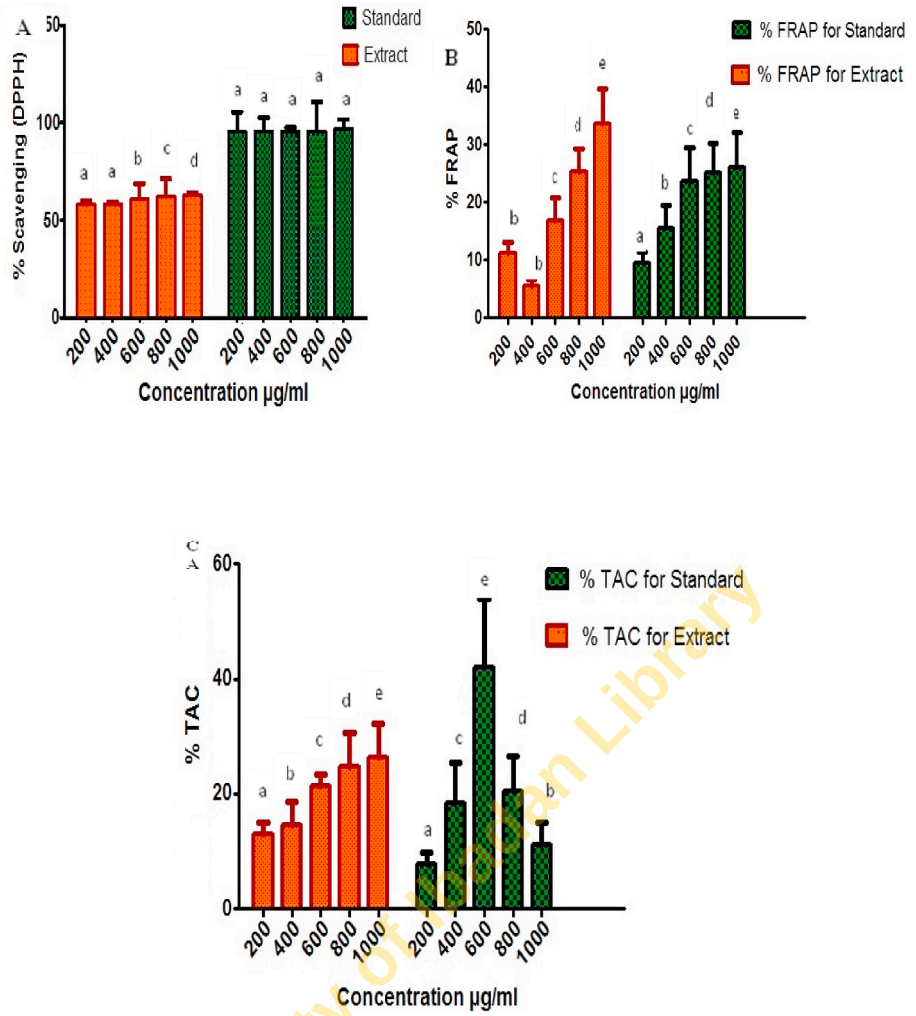

Fig. 1. *In vitro* antioxidant assays of *Ganoderma lucidum ethanol extract* (GLEE). DPPH scavenging activity (A), ferric ion reducing ability potential (FRAP) (B), and Total antioxidant capacity (TAC) (C). Values are expressed as mean ± standard error of mean (SEM). Values were recorded in triplicates (n = 3). Bars with different letters are statistically distinct ($p < 0.05$).

3.2. Proximate composition of Ganoderma lucidum ethanol extracts (GLEE)

The proximate composition of *Ganoderma lucidum* is presented in Table 4.

Proximate analyses of GLEE revealed a high amount of carbohydrate (53.68%), moisture content (17.84%), crude protein (11.79%), ash content (8.5%), crude fibre (8.16%), and fat content (0.03%).

3.3. In vitro antioxidant activities profiling of Ganoderma lucidum ethanol extract (GLEE)

The DPPH scavenging activity, ferric ion reducing ability (FRAP), and total antioxidant capacity (TAC) of GLEE were presented in Fig. 1A, B, and 1C, respectively. There was near dose-dependent increments in the DPPH scavenging activity and FRAP of GLEE. There was no significant difference ($p > 0.05$) in the DPPH scavenging activity and FRAP of GLEE at 200 µg/mL and 400 µg/mL doses. Otherwise, the activity increased dose-dependently. There was no significant difference in the DPPH scavenging activity of the standard (Ascorbate) at all doses while TAC activity was highest at the 600 µg/mL dose. TAC showed a dose-dependent increase at the doses evaluated. Concentrations of the extract and the standard (Ascorbate) evaluated are 200-, 400-, 600-, 800-, and 1000 µg/mL, respectively.

3.4. In vivo studies

3.4.1. Ganoderma lucidum ethanol extract (GLEE) modulates blood pressure components, body weight, and blood glucose of metabolic syndrome (MetS) – induced rats

As shown in Fig. 2A, MetS caused 21.90% ($p < 0.05$) increase in the systolic blood pressure relative the Normal control. Nevertheless, the combination of Glibenclamide and Atenolol reversed the systolic blood pressure by 56.72%. A dose-dependent decrements (32.01%, 34.61%, and 41.35%) ensued in the MetS groups treated with GLEE, relative to the untreated MetS group. After two weeks of treatment with GLEE, the diastolic blood pressure in the MetS untreated group showed 15.77% increase over the Normal control group. Combination of Glibenclamide and Arenolol proved more effective (74.33% decrease) in lowering the diastolic blood pressure. Notably, we observed 35.32% decrease in the MetS group treated with 26 mg/kg body weight GLEE, while 44-and 70 mg/kg body weight treated group abated the increased diastolic blood pressure by 15.75- and 21.61% respectively when compared with the untreated MetS group (Fig. 2B). Mean arterial blood pressure (MABP), is the average blood pressure in a minute-a measure of organ reperfusion (Das, 2017). GLEE, in a hormetic manner, significantly ($p < 0.05$) reversed MetS – induced increase in MABP by 26.99, 21.63, and 12.63%, respectively (Fig. 2C).

The body weight of the animals in MetS control reduced by 29.85%

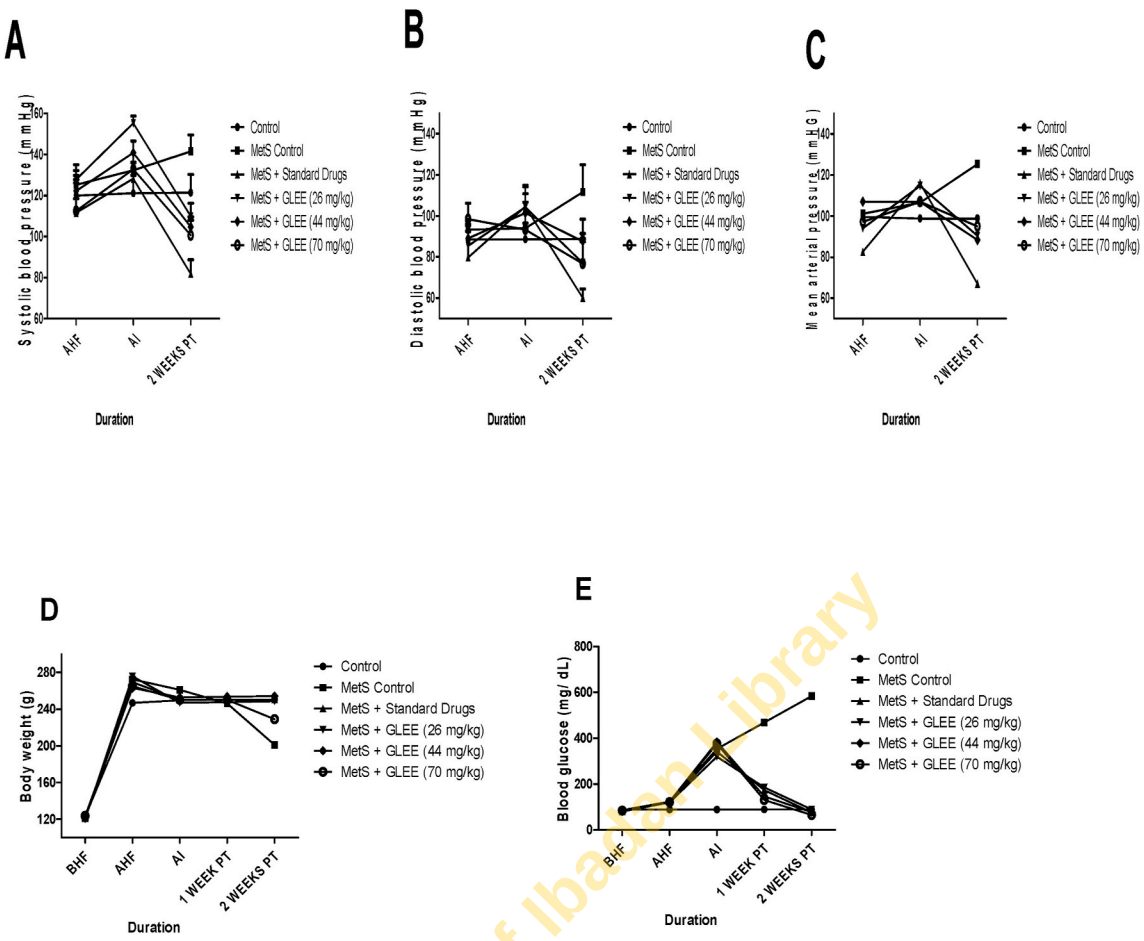

Fig. 2. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on blood pressure components [systolic blood pressure (A), diastolic blood pressure (B), and mean arterial pressure (C) (mmHg)] and body weight (D) (g) and blood glucose (E) (mg/dL) of the rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5); $p < 0.05$ is considered significant. BHF- Before feeding with High Fat Diet, AHF- After feeding with High Fat Diet and administration of 10% fructose, AI- After injection of Streptozotocin 1 WEEK PT- One week post-treatment with GLEE, and 2 WEEKS PT- Two weeks post-treatment with GLEE.

at the end of the two weeks GLEE treatment relative to the Normal control (Fig. 2D). Notable increases was observed in MetS +26 mg/kg BW GLEE group (0.72%), more than 0.55% observed in the MetS + 44 mg/kg BW GLEE group when compared with the MetS control. GLEE essentially reversed MetS induced hyperglycemia in a dose-dependent manner up to 4-folds in the MetS +70 mg/kg BW GLEE, when compared with the MetS control where there was 40% increase in blood sugar when compared with the normal control (Fig. 2E). The combined effect of Glibenclamide and Atenolol caused about 3.4–folds decrease in the hyperglycemia is comparable to the effect produced in the MetS +44 mg/kg BW GLEE group where 3.9-fold decrease ensued.

3.4.2. Metabolic syndrome (MetS) – induced dyslipidemia was abated due to Ganoderma lucidum treatment

Plasma levels of triacylglycerol (TAG), cholesterol (CHOL), and low-density lipoprotein cholesterol (LDL- CHOL) were markedly ($p < 0.05$) in the MetS control group when compared with the normal control. Nevertheless, the three treated groups (i.e. MetS +26-, 44-, and 70 mg/kg) showed a remarkable decrease in the CHOL and LDL- CHOL levels regardless of the dose. MetS – induced hypertriglyceridemia was abated completely by the two lower doses of GLEE. There was no significant difference in the level of plasma high-density lipoprotein (HDL) in all the groups (Fig. 3A, B, C, and D).

3.4.3. Ganoderma lucidum ethanol extracts (GLEE) normalizes the in vivo antioxidant status and mollifies oxidative damage marker in rats

There was significant ($p < 0.05$) increments of 13.76%, 1. 4 fold, 15.5%, and 30.57% due to induction of MetS in the SOD of the pancreas, heart, liver, and kidneys of the rats respectively when compared with the Normal control. GLEE at 70 mg/kg dose proved more effective in abating the increments by causing reductions in the pancreas (33.11%), heart (26.97%), liver (13.69%), and 25.16% in the kidneys when compared with the MetS control (Fig. 4A, B, C, and D).

CAT activity increased significantly ($p < 0.05$) in the pancreas and the heart but decreased in the liver and kidney of the MetS control animals when compared with the Normal control. Decrements of 29.31% and 33.63% in CAT activity were recorded in the pancreas and heart respectively in MetS control group. However, treatment with 70 mg/kg GLEE caused 9.33% decrease in the pancreas, and 31.06% decrement in the heart relative to the MetS control group (Fig. 5A and B). Intriguingly, the liver CAT activity decreased by 30.21%, and kidney CAT activity was down by 25.22% in the MetS control group. Nevertheless, GLEE (MetS + 70 mg/kg) showed a tremendous ability to enhance CAT activity by increasing the CAT activity up to 1.66 fold in the liver and 26.42% in the kidneys (Fig. 5C and D) when compared with the MetS control group.

MDA level measures the extent of lipid peroxidation. We measured MDA level in the pancreas (Fig. 6A), the heart (Fig. 6B), the liver (Fig. 6C), and the kidneys (Fig. 6D). MetS group showed 54.2, 52.9, 2.75 folds, and 34.8% increase in MDA level as estimated in the pancreas, heart, liver, and kidneys respectively. However, treatment with GLEE

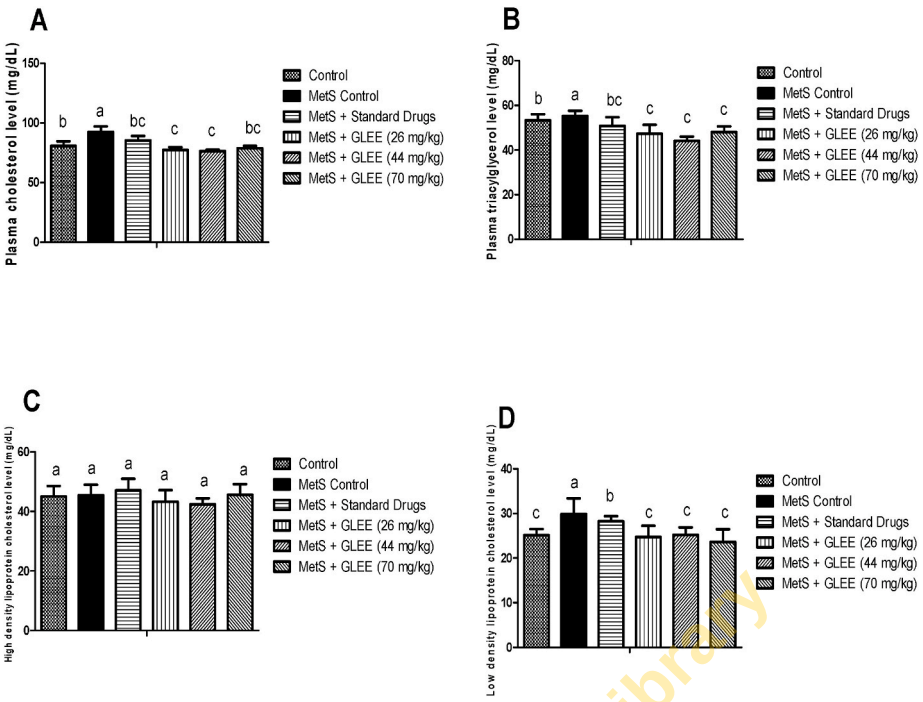

Fig. 3. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on plasma lipid profiles [cholesterol (A), triacylglycerol (B), high density lipoprotein (C), and low density lipoprotein (D) (mg/dL)] of rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5). Bars with different letters are statistically distinct ($p < 0.05$).

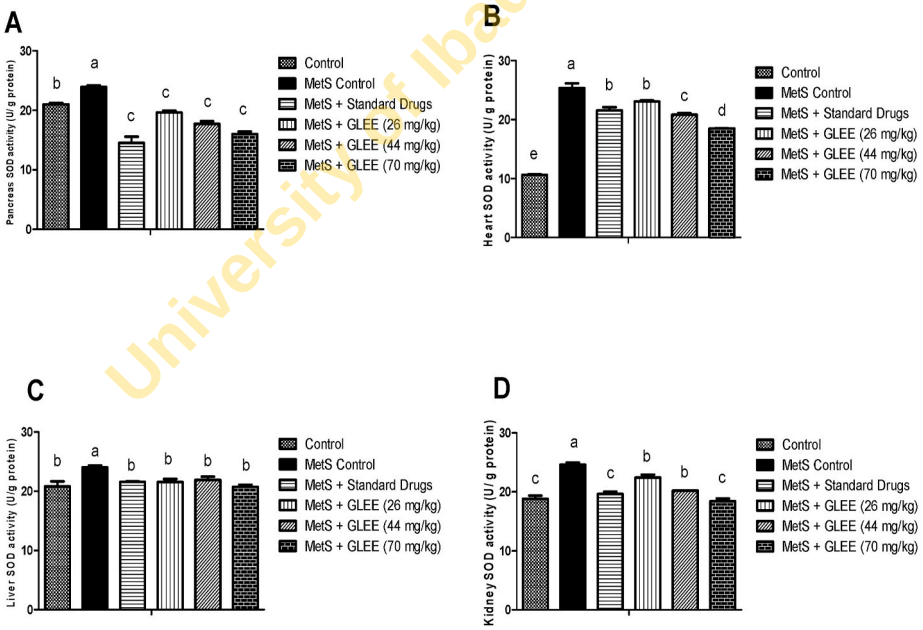

Fig. 4. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on tissues [Pancreas (A), Heart (B), Liver (C), and Kidney (D) (U/g protein)] superoxide dismutase (SOD) activity of rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5). Bars with different letters are statistically distinct ($p < 0.05$).

essentially abated the increased MDA level observed in the MetS control animals by 37% in the pancreas, 65.58% in the heart, 43.17% in the liver, and 73.2% in the kidneys. Although, other doses also produced desired effects, the 70 mg/kg body weight dose of GLEE was the most effective.

Fig. 7 shows that, the NRF2 level in the pancreas, heart, liver, and kidney increased significantly ($p < 0.05$) in the MetS control relative to the Normal control. However, treatment with GLEE normalized the NRF2 level in the pancreas, liver, and kidney. GLEE treatment reduced the NRF2 level in a dose-dependent manner in the kidney, whereas, there was no significant difference in the level of NRF2 for the three doses of GLEE in the pancreas, and the liver. Neither the standard drug nor GLEE was able to reduce the NRF2 level in the heart.

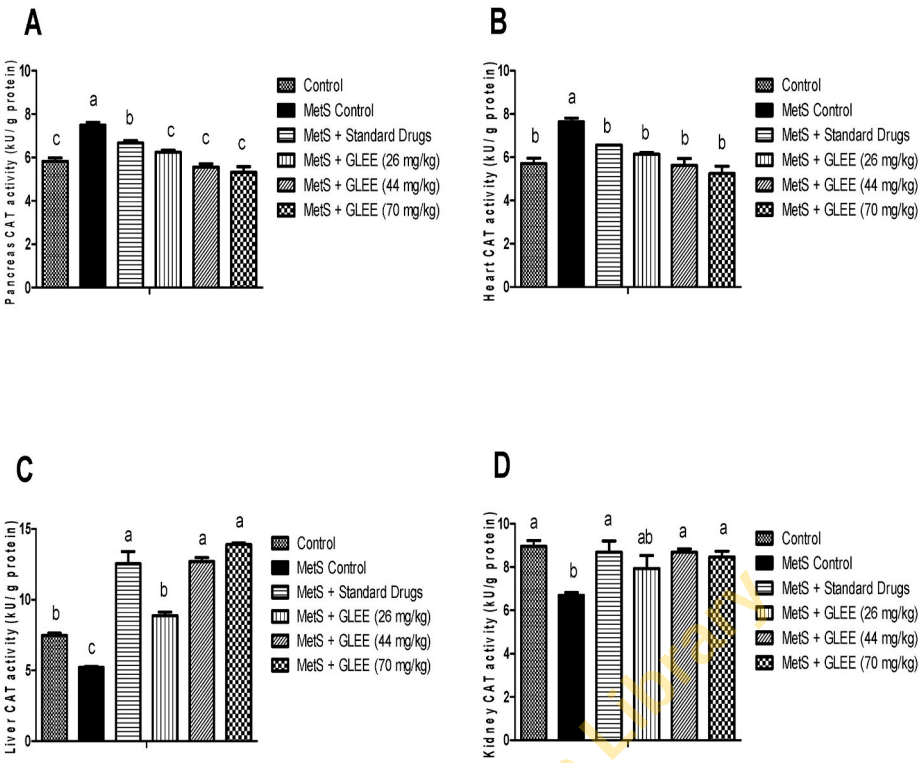

Fig. 5. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on tissues [Pancreas (A), Heart (B), Liver (C), and Kidney (D) (U/g protein)] catalase (CAT) activity of rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5). Bars with different letters are statistically distinct ($p < 0.05$).

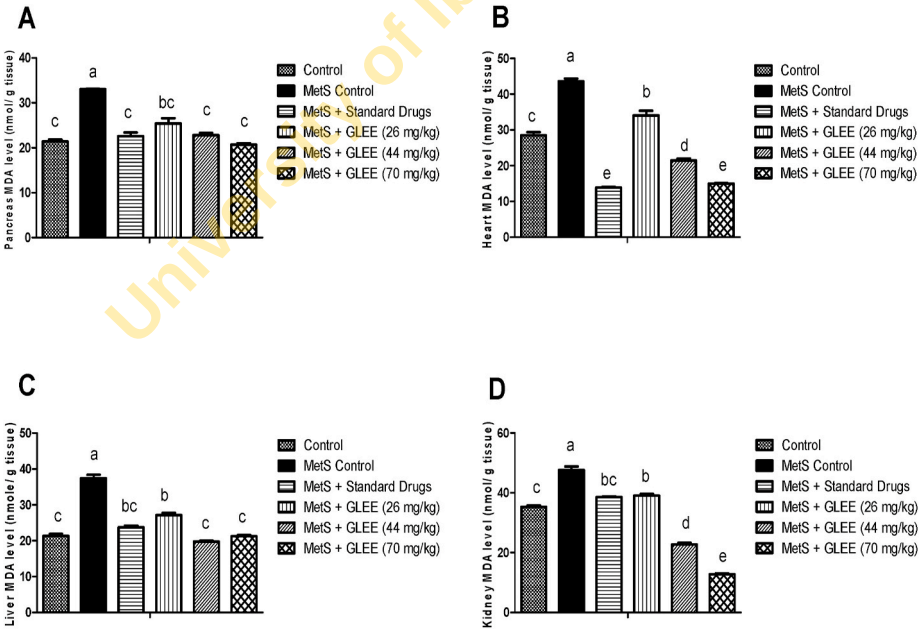

Fig. 6. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on tissues [Pancreas (A), Heart (B), Liver (C), and Kidney (D) malondialdehyde (mmol/g tissue)] level of rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5). Bars with different letters are statistically distinct ($p < 0.05$).

3.5. GLEE ameliorated MetS – mediated derangement in the histo-morphological structure of the pancreas, heart, liver, and kidneys

CAT activity increased significantly ($p < 0.05$) in the pancreas and the heart but decreased in the liver and kidney of the MetS control animals when compared with the Normal control. Decrements of 29.31% and 33.63% in CAT activity were recorded in the pancreas and heart respectively in MetS control group. However, treatment with 70 mg/kg GLEE caused 9.33% decrease in the pancreas, and 31.06% decrement in the heart relative to the MetS control group (Fig. 5A and B). Intriguingly, the liver CAT activity decreased by 30.21%, and kidney CAT activity was down by 25.22% in the MetS control group. Nevertheless, GLEE (MetS

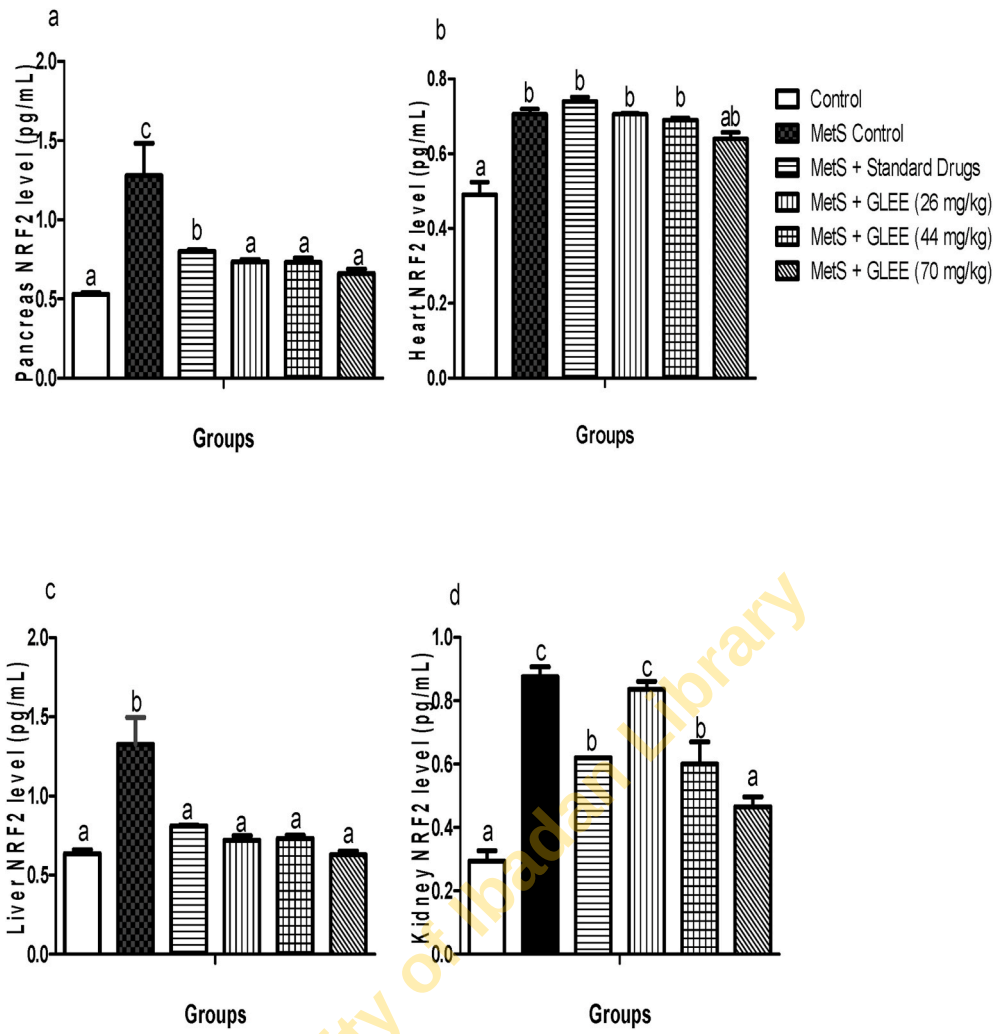

Fig. 7. Effects of *Ganoderma lucidum ethanol extract* (GLEE) on tissues [Pancreas (A), Heart (B), Liver (C), and Kidney (D) NRF2 protein level (pg/mL)] level of rats induced with metabolic syndrome (MetS). Values are expressed as mean ± standard error mean (SEM) (n = 5). Bars with different letters are statistically distinct ($p < 0.05$).

+ 70 mg/kg) showed a tremendous ability to enhance CAT activity by increasing the CAT activity up to 1.66 fold in the liver and 26.42% in the kidneys (Fig. 5C and D) when compared with the MetS control group.

4. Discussion

Studies have shown that plants, fungi, or animal-derived fibres possess an anti-obesity effect by different mechanisms (Chen et al., 2020). Ganoderma lucidum is popularly used in the Asian countries since antiquity, due to report of an array of beneficial health effects such as anti-inflammatory and immunomodulatory effects (Gao et al., 2004). Besides, attenuation of cardiovascular risk factors such as hypertension, hyperglycemia, and dyslipidemia are other effects (Klupp et al., 2016). Undoubtedly, MetS remain a global health concern due to the progressive upsurge in the prevalence of obesity, type 2 diabetes, and hypertension (Klupp et al., 2016). According to the World Health Organisation, metabolic syndrome clusters under five components; hypertension, copious hyperglycemia, reduced HDL-cholesterol, and central obesity (Mulè et al., 2014). An individual is said to have MetS if they exhibit up to three or four components (Mule et al., 2014). Because MetS is an array of these mentioned related but independent factors, management of the complications arising from MetS has since focused on individual factor rather than the cluster. Although, lifestyle modifications can limit the modifiable risk factors, the inability to sustain such lifestyles has made pharmacological intervention a must for individuals to attenuate the complications from MetS. There is currently no approved single drug for the management of MetS because of its multifactorial nature (Klupp et al., 2016). Therefore, a suitable candidate drug must be capable of abating at least four components of MetS. This study, therefore, evaluated the potential efficacy (hypotensive, anti-hyperglycemic, anti-dyslipidemia, and antioxidant effects) of Ganoderma lucidum ethanol extract (GLEE) in the management of metabolic syndrome (MetS) in the murine model.

Phytochemical analyses in our study shows that GLEE possesses alkaloids, flavonoids, saponins, and terpenoids et al. Proximate analysis also showed that carbohydrate is the main component (53%), and fibre content is about 8.16%, the fat content is low (0.03%). Our observations align with that of Salvatore et al. (2020) who showed that the main components of Ganoderma lucidum (G. lucidum) are triterpenes, polysaccharides, and triglycerides. The pharmacologic effects of G. lucidum have been adduced to their abundance of polysaccharides and terpenoids (Klupp et al., 2016). Likewise, *Cordyceps Sinensis*, a similar medicinal plant of Chinese source has been reported to contain a panoply of polysaccharides responsible for its anti-obesity and hepato-renal protective effects (Chen et al., 2020).

To evaluate the in vitro antioxidant activity of GLEE, the extracts were subjected to in vitro antioxidant assays. Our results show that total antioxidant capacity (TAC) of GLEE increased in a dose-dependent

manner whiles the ferric ion reducing ability potential (FRAP) and DPPH radicals scavenging ability showed a near dose-dependent increase (exception is at 200 μL/mL). This ability of GLEE to reduce and or scavenge radicals have been reported by Rajasekaran and Kalaimagal (2011) that GLEE showed a dose-dependent increase in the ability to scavenge DPPH radicals and reduction of ferric radicals in vitro.

The combined effects of high-fat diet feeding, fructose water and single intraperitoneal injection of streptozotocin were capable of eliciting at least, four of the components of MetS in our study. Rats exhibited hyperglycemia, dyslipidemia, diabetes, and hypertension progressively although; there was no significant difference in high-density lipoprotein (HDL) cholesterol level in all the experimental groups. The reason for this remains unclear. However, a similar result was obtained in a randomized placebo-controlled subject as reported by Klupp et al. (2016), where there was no significant difference between the MetS subject and those treated with G. lucidum.

The occurrence of hypertension in MetS patients seems to be an 'unholy matrimony' as indicated by Mule et al. (2014). Undoubtedly, hypertension tends to cluster with cardio-metabolic and anthropometric derangements usually observed in MetS patients and is centrally involved in all the components of MetS in several ways. For instance, it has been shown that hypertension shows positive correlations with insulin resistance, dyslipidemia, and obesity (Mendizabal et al., 2013). Furthermore, drugs used against insulin resistance (IR) also to possesses anti-hypertensive effects (Mule et al., 2014). Alternatively, antihypertensive drugs such as angiotensin-converting enzyme II (ACE II) inhibitor increases insulin sensitivity (Mendizabal et al., 2013). In our study, progressive hypertension was characterized by elevated systolic, diastolic, and mean arterial blood pressure in the Mets control group, relative to the Normal control (Fig. 2 A, B, and C). GLEE appeared to show an antihypertensive effect in the MetS but treated groups. The highest dose (70 mg/kg) of GLEE showed the most significant activity, thereby, validating our earlier in vitro study, which shows dose-dependent beneficial responses. Our observation is consistent with a clinical trial reported by Klupp et al. (2016) who reported a significant decrease in the mean arterial blood pressure (MABP) of subjects treated with G. lucidum. A novel triterpene; trametenolic acid, in conjunction with ergosterol possesses anti-cardio-metabolic risk effects in vivo (Atenules et al., 2020). Therefore, the bioactivity of GLEE against MetS – induced hypertension might be due to the presence of these terpenoids. GLEE also showed a tremendous ability to regulate the bodyweight of the experimental animals. As indicated in Fig. 2D, feeding the animals with a high-fat diet and fructose water invoked a dramatic weight gain akin to an overweightedness associated with type 2 diabetic individuals. However, following STZ injection, the MetS control group continuously experienced a decrease in body weight. Although, the standard drugs and the GLEE maintained the animals weight, the lowest dose of GLEE (26 mg/kg) caused a near-normal weight thereby preventing organ failure that could result from the reduced weight (Fig. 2D). Corroborating this organ failure sparing effect is that, elevated MABP values correlate positively with organ failure. MABP at Normal value (60–80 mmHg) is necessary for proper organ perfusion but, increased value is diagnostic of kidney failure (Das, 2017).

As expected, blood sugar level increased copiously after the induction of diabetes, and the hyperglycemia progressively increased in the untreated MetS group relative to Normal control (Fig. 2E). Hyperglycemia and hyperinsulinemia are commonplace in MetS patients (Mule et al., 2014). Although, we did not measure the plasma insulin in the current study, progressive hyperglycemia observed in the untreated MetS control suggests that the animals are insulin resistant. It may be safe to say that the insulin hypothesis of hypertension is playing out here. The insulin hypothesis proposed that the compensatory hyperinsulinemia in the insulin-resistant subjects might increase the Na + reabsorption and sympathetic activity in the renal cells. The cumulative effect of this increment is the elevation of the arterial blood pressure, and consequently, hypertension (Mendizabal et al., 2013).

Dyslipidemia is associated with an excessive blood TAG, cholesterol (CHOL), and other lipids levels. In the present study, the GLEE dose-dependently reduces the MetS – induced elevation in the TAG, CHOL and the low-density lipoprotein (LDL) – cholesterol level in the treated group, when compared with the untreated group (Fig. 3 A, B, and C). There was no significant difference in the HDL-cholesterol levels, as mentioned earlier. The elevated lipid level is consistent with other findings (Chen et al., 2020; Klupp et al., 2016) typical of MetS conditions. Hyperglycemia and hyperlipidemia are two of the three metabolic diseases that exacerbate a sympathetic tone. Indeed, high circulating free fatty acid due to excessive lipolysis may in turn activate the sympathetic system and leads to hypertension (Mendizabal et al., 2013). The ability of GLEE to modulate the lipids level might be due to the presence of fibres, which may delay intestinal lipid absorption and modulate the activity of cholesterogenic enzymes (Antunes et al., 2020). Furthermore, according to Lu et al. (2010), the presence of Beta-glucan in G. lucidum is probably responsible for its cholesterol and postprandial hyperglycemia lowering effects.

Substantive experimental evidences have linked oxidative stress to a myriad of pathological conditions including MetS (Chung et al., 2003; Ugbaja et al., 2020). Undoubtedly, oxidative stress plays a significant role in the aetiology of nearly all the components of MetS. Hyperglycemia may induce oxidative stress via the polyol pathway by depleting the reduced GSH level (Aldose reductase reaction) and elevation of advance glycated end-products (AGEs) (Chung et al., 2003). The beta-cell of the pancreas may be destroyed by peroxynitrite radical (combination of nitric oxide with molecular oxygen) and DNA damage mediated by poly ADP- ribose polymerase activation (Gao et al., 2000). Furthermore, deposition of oxidized LDL in the arterial wall may cause atherosclerosis and microvascular dysfunction that may cause hypertension (O'Neill and O'Driscoll, 2015). Bearing this in mind, we evaluated key antioxidant enzymes activities; superoxide dismutase (SOD) and catalase (CAT) in the pancreas, heart, liver, and kidney due to their various important and distinct metabolic roles. Moreover, the level of MDA was estimated in like manner. SOD activities (Fig. 4) and MDA levels (Fig. 6) increased significantly in the MetS untreated group as expected. CAT activities increased in the pancreas and heart but reduced strangely in the liver and kidneys (Fig. 5). SOD catalyses the dismutation of toxic superoxide radicals to hydrogen peroxide, which is then broken down by CAT or glutathione peroxidase (GPx) to water and oxygen (Cervantes Gracia et al., 2017; Ugbaja et al., 2020). Failure of the aforementioned antioxidant mechanism may invoke the abstraction of electrons from the electron-rich biomolecules such as polyunsaturated fatty acids. The abstractions of electrons then initiate the vicious lipid peroxidation cascade and culminate in the production of highly reactive MDA (Cervantes Gracia et al., 2017). GLEE essentially abolished the increased SOD activity and MDA level in the pancreas, heart, liver, and kidney respectively. CAT activity was also normalized accordingly. To elucidate the involvement of oxidative stress and the antioxidant system in the aetiology of MetS, we determined the protein level of NRF2 due to its modulation of the cellular antioxidant response. NRF2 plays critical regulatory and cytoprotective role against oxidative stressors (Chartoumpekis and Kensler, 2013). Our results show that NRF2 level increased significantly in the MetS group, relative to the control group. Perhaps the increased NRF2 level is responsible for SOD and CAT increased activities in this study. In an attempt to evaluate the role of NFR2 in the incidence of insulin resistance and metabolic syndrome, conflicting results have been obtained so far (Li et al., 2020). Chartoumpekis et al. (2011) showed that Nrf2-KO mice were protected partially from insulin resistance following a chronic exposure to a high-fat diet. Contrastingly, Yu et al. (2011) showed that Nrf2-KO mice gained more weight than the wild type when maintained on a high-fat diet, and does not prevent obesity. Increased NFR2 in the pancreas, heart, liver, and kidney of MetS group as seen in this study might be associated with increased inflammatory response as metabolic syndrome correlates positively with induction of inflammation (Li et al.,

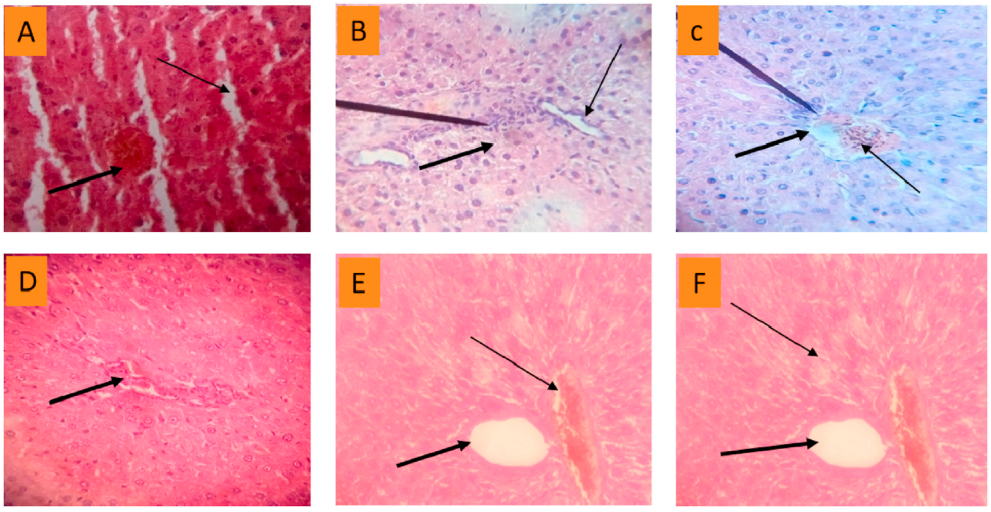

Fig. 8. Photomicrograph slides of rat liver (Hematoxylin and eosin staining, 100x magnification). (A) Control shows normal hepatic architecture with mild hyperemia (thick arrow). (B) MetS control liver showed congestion of the portal and central area (pointed pin), necrosis of the mid-zone (thin arrow), and infiltration of the inflammatory cells (thick arrow). (C) MetS + standard drug shows congested portal veins (thick arrow) and central vein (thin arrow and pin head). (D) MetS + 26 mg/kg GLEE rats show mild congestion of the central area and mild necrosis (thick arrow). (E) MetS + 44 mg/kg GLEE group shows mild hyperemia (thin arrow) and mild necrosis of the parenchyma tissue (thick arrow). (F) MetS + 70 mg/kg GLEE rats show mild hyperemia and congestion of the central vein (thick arrow), and mild edema of the zone 2 (thin arrow).

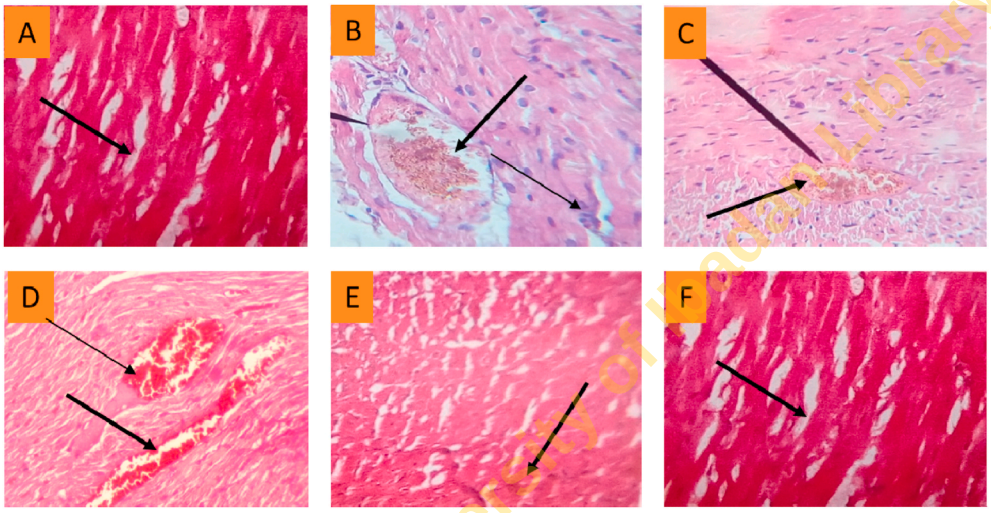

Fig. 9. Photomicrograph slides of rat heart (Hematoxylin and eosin staining, 100x magnification). (A) Control shows normal cardiac architecture. (B) MetS control rats showed congestion of the parenchyma (pointed pin), mild necrosis of the mid-zone (thin arrow), and infiltration of the inflammatory cells (thick arrow). (C) MetS + standard drug shows congested parenchyma (thick arrow) and infiltration of inflammatory cells (pin head). (D) MetS + 26 mg/kg GLEE rats show mild congestion of the coronary vein (thin arrow) and haemorrhage of the perimysium (thick arrow). (E) MetS + 44 mg/kg GLEE group shows mild hyperemia of the myocardium (thick arrow) (F) MetS + 70 mg/kg GLEE rats show normal myocardium architecture.

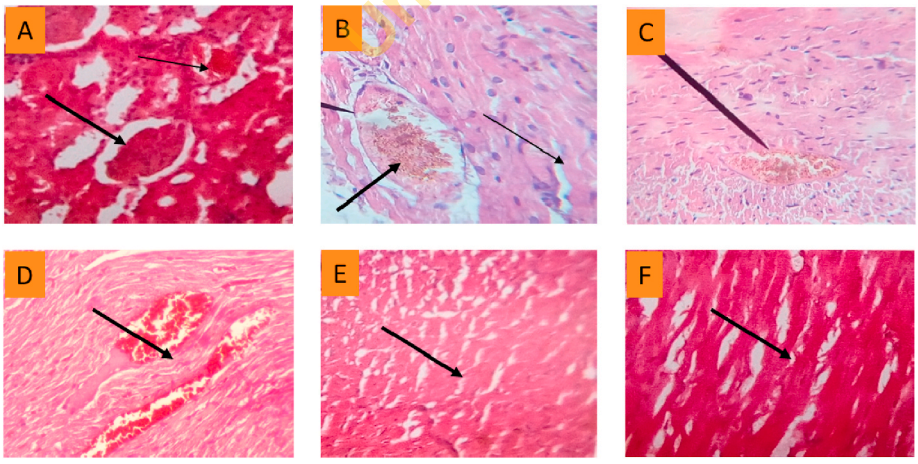

Fig. 10. Photomicrograph slides of rat kidney (Hematoxylin and eosin staining, 100x magnification). (A) Control kidney shows no pathological feature. (B) MetS control rats showed tubular necrosis (pointed pin), corticomedullary junction congestion (thin arrow), and infiltration of the inflammatory cells (thick arrow). (C) MetS + standard drug shows mild congestion of cortex parenchyma (thick arrow) and infiltration of inflammatory cells (pin head). (D) MetS + 26 mg/kg GLEE rats had dilated medullar at the distal end of the convoluted tubule (thin arrow) (E) MetS + 44 mg/kg GLEE group shows mild hyperemia and congestion of the corticomedullary junction (thick arrow). (F) MetS + 70 mg/kg GLEE rat shows no pathological feature.

2020). Regardless, treatment with GLEE abated the aberrant increment of NFR2 in the pancreas, liver, and kidney. Intriguingly, GLEE did not produce a statistically significant result in the heart. The effect appeared to be more pronounced in the pancreas, liver, and kidneys-the organs with a more specified role for endocrine and regulatory effects. Accordingly, the antioxidant activity of GLEE might be because of the presence of flavonoids, as confirmed by our in vitro analyses. Flavonoids are known for their electron-donating effect and radicals scavenging

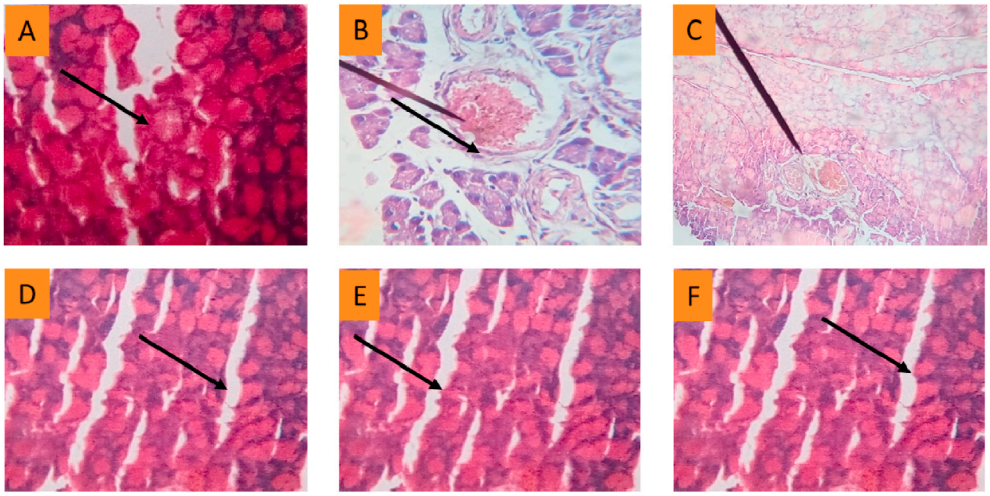

Fig. 11. Photomicrograph slides of rat pancreas (Hematoxylin and eosin staining, 100x magnification). (A) Control kidney shows no visible lesion. (B) MetS control rats showed congestion of the pancreatic parenchyma (thick arrow), and infiltration of the inflammatory cells (pin head). (C) MetS + standard drug shows mild edema and congestion of the parenchyma (pin head). (D) MetS + 26 mg/kg GLEE rats show no pathological lesion (E) MetS + 44 mg/kg GLEE group shows normal pancreatic architecture. (F) MetS + 70 mg/kg GLEE rat shows no pathological feature (Krik et al., 2008) (AOAC, 2010) (Shi et al., 2011).

activities probably due to the presence of multiple hydroxyl groups which is responsible for their health benefits (Chen and Raji, 2020).

Due to the systemic effects of MetS, the derangement of metabolic organs histopathology is a commonplace (Theurer et al., 2020). We therefore investigated the GLEE effects on MetS –induced histopathology of the pancreas (Fig. 7), heart (Fig. 8), liver (Fig. 9), and kidney (Fig. 10). MetS invoked a range of derangements to the histology of the rat organs. The untreated MetS control showed extensive necrosis, cellular degeneration, and massive infiltration of the inflammatory cells. The groups treated with GLEE, however (especially the highest dose) showed normal cytology devoid of any pathological features. Fig. 11.

4.1. Conclusion

Taken together, GLEE (70 mg/kg body weight) showed tremendous biological effects, characterized by in vitro and in vivo antioxidant activities, antihypertensive, anti-hyperglycemic, and anti-dyslipidemia effects in a rat model of metabolic syndrome. Besides, NRF2 protein level, which was aberrantly increased in the MetS group, was significantly reduced in the GLEE-treated groups, indicating the modulatory role of GLEE against hyper-activation of the antioxidant responsive pathways. We also found that, rats treated with GLEE did not show any pathological feature in the pancreas, heart, liver, and kidneys. This study therefore showed that Ganoderma lucidum might be a candidate regimen in the management of MetS.

CRediT authorship contribution statement

Akindele Oluwatosin Adeyi: Methodology, Supervision. **Shakirat Adedoyin Awosanya:** Project administration, Writing - original draft. **Olubisi Esther Adeyi:** Data curation, Writing - review & editing. **Adewale Segun James:** Writing - original draft, Writing - review & editing, Project administration. **Clementina Oyinkansola Adenipekun:** Conceptualization, Supervision.

Declaration of competing interest

We declare no conflict of interest for this study.

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