Toxicology Reports 11 (2023) 177–188

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Drying temperature affects the hypolipidemic, antioxidant, and antihypertensive potential of *Hibiscus sabdariffa* calyx in rats induced with L-NAME

John Osarenren Efosa [a], Kingsley Omage [b,c,*], Marshall Arebojie Azeke [d]

[a] Department of Physical Laboratory Technology, School of Applied Sciences and Technology, Auchi Polytechnic, Edo State, Nigeria
[b] Department of Biochemistry, College of Basic Medical Sciences, Igbinedion University Okada, Edo State, Nigeria
[c] Division of Nephrology and Hypertension, Department of Medicine, Oregon Health and Science University, Portland, OR, USA
[d] Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Edo State, Nigeria

ARTICLE INFO

Handling Editor: Dr. L.H. Lash

Keywords:
Lipid profile
Oxidative stress
Cardiovascular diseases
Blood pressure
Hibiscus sabdariffa calyx

ABSTRACT

The effects of different drying temperatures on the hypolipidemic, antioxidant, and antihypertensive potential of *Hibiscus sabdariffa* calyx was evaluated. The calyx were dried under different temperature conditions (− 58 °C, 30 °C, 40 °C, and 50 °C), and extracted with a solvent mixture of ethanol and water (1:4 % w/v). To induce hypertension, the rats were administered with 40 mg/kg body weight dose of N-nitro L-arginine methyl-ester (L-NAME), via the intra-gastric route. *H. sabdariffa* extract was administered orally, at varying doses (250, 500, and 1000 mg/kg) to the rats. Afterwards, the hypolipidemic, antioxidant, and antihypertensive potentials of the extracts were evaluated using standard validated methods. Induction with L-NAME significantly ($p < 0.05$) increased the total cholesterol, triglyceride, and LDL levels, significantly decreased the HDL levels; significantly ($p < 0.05$) increased the levels of LPO/MDA, H_2O_2, and decreased GPx, and SOD activities; significantly ($p < 0.05$) increased the pressures (diastolic and systolic); significantly ($p < 0.05$) increased ACE and arginase activities, glucose level, and significantly decreased nitric oxide activity. Treatment with *H. sabdariffa* extract significantly ($p < 0.05$) reversed these trends in the hypertensive experimental rats. The hypolipidemic, antioxidant, and antihypertensive properties of the extract from the calyx of *H. sabdariffa*, which varies with the drying temperatures of the calyx, portends its potential as a curative agent in the treatment of hypertensive conditions, and other cardiovascular diseases.

1. Introduction

Among cardiovascular diseases (CVDs), hypertension is the most common, and has become a notable health challenge in countries all over the world, whether developed, or developing [1,2]. Principally, it increases the chances of developing several diseases like aneurysm, heart attack, stroke, heart failure, and other diseases of cardiovascular origin [2]. Previously, oxidative stress was demonstrated to play a vital role in the processes which lead to the development of hypertension [3]. In fact, hypertensive patients have been reported to exhibit marked increase in oxidative stress [4]. Newly diagnosed and untreated hypertensive subjects, reportedly show decrease in the activities of glutathione peroxidase and superoxide dismutase, which inversely correlates with blood pressure [4]. It has also been reported that hydrogen peroxide and lipid peroxide production increased in hypertensive subjects [5]. However, it has been reported that naturally occurring antioxidants, from food or medicinal plants, are capable of fighting oxidation stress by stabilizing the levels of antioxidant activities in the biological system [6–9]. Several reports have also demonstrated that naturally occurring antioxidants from plant sources could be formulated as nutraceuticals for thwarting the course of hypertension, target organ impairment, and other diseases of cardiovascular origin [10–13].

Inhibitors of the angiotensin I-converting enzyme (ACE) are also commonly used in managing hypertension, because they are known to be useful in decreasing the death rates among hypertensive patients [14]. In comparison with drugs derived from chemosynthetic compounds, inhibitors of the ACE that are obtained from natural sources, like medicinal plants or food proteins, are perceived to possess less

* Corresponding author at: Department of Biochemistry, College of Basic Medical Sciences, Igbinedion University Okada, Edo State, Nigeria.
E-mail addresses: omagekingsley@yahoo.com, omage.kingsley@iuokada.edu.ng (K. Omage).

https://doi.org/10.1016/j.toxrep.2023.09.005
Received 27 July 2023; Received in revised form 25 August 2023; Accepted 5 September 2023
Available online 6 September 2023

adverse effects [15]. These inhibitors from plant sources normally behave like vasodilators, because they often bring about the reduction of the concentration of angiotensin II in the rennin-angiotensin system. It is worthy of note that the function of the renin–angiotensin-aldosterone system (RAS), in the control of blood pressure and maintenance of sodium homeostasis, is physiologically very important. It regulates the pressure of the blood by increasing the rate of reabsorption of sodium, and the retention or reabsorption of water, as well as the constriction of the blood vessels [16]. Previously, the extracts from some medicinal plants, like *Acalypha wilkesiana, Hibiscus sabdariffa,* have been shown to demonstrate antihypertensive activity [17] by at least three major and specific mechanisms of action: diuretic [18], vasodilator [19], and angiotensin converting enzyme inhibition [20]. The maintenance of the homeostasis of body fluid and blood pressure also involve the actions of nitric oxide (NO) [21]. In fact, certain diseases, like essential hypertension, have long been associated with diminished synthesis of NO, or its action [22]. Thus, the use of an orally-active inhibitor of nitric oxide synthase (NOS), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), to bring about the chronic inhibition of basal NO, serves as a good experimental model of hypertension. L-NAME, when administered chronically, could result in significant increases in the blood pressure in experimental animals. The resulting experimental hypertension often exhibit typical pathologic and physiologic features that are similar to that of primary or essential hypertension, due to its associated increase in blood pressure without a perceptible underlying cause [22].

It is a common practice in traditional medicine for mixtures of the components of plants to be specified as a curative measure for hypertension, and some other forms of CVDs [23–25]. Many plants containing alkaloids and flavonoids exhibit diuretic, anti-oxidant, anti-inflammatory, antispasmodic, and analgesic potentials [26–29]. These medicinal plants have found wide use as curative agents in the management of hypertension, as well as other CVDs [1,30,31]. *Hibiscus sabdariffa,* a fleshy calyx, commonly used for the production of wine, and a variety of other beverages [32], is a popular herbal plant that is often used in traditional medical practice. A number of phytochemicals, with bioactivities as antioxidants, and antihypertensive agents, are reportedly abundant in *H. sabdariffa* [33]. For example, the anthocyanins contained in *H. sabdariffa* has been found to possess antioxidant activity, protects against atherosclerosis, and also enhance cholesterol metabolism [34]. However, these therapeutic potentials of *H. sabdariffa* may be affected by the drying temperature of the calyx. Different drying temperatures have been shown to affect the availability, and potencies of some of these phytochemicals that are found in *H. sabdariffa* [33]. Thus, it is very important to determine which temperature is best to dry *H. sabdariffa,* specifically the calyx, for maximum utilization of its therapeutic potentials. Taking this into consideration in this study, we prepared different extracts from *H. sabdariffa* calyx (after drying at varying temperatures), and evaluated its hypolipidemic, and antioxidant properties, as well as its ability to ameliorate the deleterious effects of hypertension. This was done using experimental rat models that were administered with L-NAME (an agent which is capable of inducing experimental hypertension).

2. Materials and methods

2.1. Preparation of dried powdered samples of H. sabdariffa calyx and its extraction

H. sabdariffa calyx were freshly harvested, from Uchi which is located in Auchi, a town located within Etsako West LGA of Edo State, Nigeria. Verification of the taxonomy of the fresh calyx was carried out at the Botany department, within the Faculty of Life Science of the Ambrose Alli University, Nigeria. After the taxonomical verification, the *H. sabdariffa* calyx were properly washed and rinsed in distilled water, and afterwards divided into four batches of about 1 kg each. They were subsequently subjected to drying at varying temperatures (i.e., − 58 °C, 30 °C, 40 °C, and 50 °C). Vacuum freeze dryer (YK-118-50, Taiwan) was used to achieve drying at − 58 °C, while an oven (SM-9023, USA) was used to achieve drying at other temperatures. After drying, the calyx were milled into a powdery form, in an aseptic condition, using an attrition mill (Lister Inc. England). The powdered samples of the calyx were subsequently weighed in sterile glass flasks. They were then dissolved in a solvent mixture of ethanol and water (1:4 % w/v), with the flasks properly sealed, and placed on a magnetic stirrer for 72 h. The extraction process was carried out at room temperature (25 ± 2 °C), with minimal agitation of the content of the flask. After extraction, excess solvent was filtered and separated by re-circulation, after which the extracts were concentrated with the aid of a rotary evaporator (RE201D).

2.2. Phytochemical screening of the extracts of the calyx of H. sabdariffa

Previous, we screened for selected phytochemical constituents in the extracts from the calyx of *H. sabdariffa* (after drying at varying temperatures), using previously described standard procedures. The results reported the absence of steroids, and coumarin glycosides, at a drying temperature of 50 °C. At 30 °C, we found significantly ($p < 0.05$) elevated quantities of phenols (93.50 ± 3.88 mgQAE/g), flavonoids (42.22 ± 1.08 mgQE/g), alkaloids (284 ± 1.08 mgQE/g), tannin (100.77 ± 0.50 mgTAE/g), and saponin (570.44 ± 0.30 mgQE/g) [33].

2.3. Animal and ethical statement

This study utilized ninety (90) male albino rats (8 weeks, weighing 150 ± 5 g). They were accommodated under hygienic conditions using plastic cages. The cages were kept in a well aerated animal facility. The rats were exposed to a natural photoperiod of 12 h-light-dark cycle. They were nourished with a standard rat feed, and allowed easy accessibility to clean drinking water, ad libitum. Before commencing the experiment, the climatic adaptation of the rats in the animal facility was allowed for 7 days. The care, handling, and work with the rats were done with strict adherence to the 'Guide for the Care and Use of Laboratory Animals', as described by the Animal Use and Care Committee (AUCC) of Ambrose Alli University. Also, the protocols used were approved by the National Health Research Ethic Committee, Nigeria.

2.4. Induction of hypertension and experimental design

The induction of experimental hypertension was done by the intragastric administration of 40 mg/kg body weight daily dose of N-nitro L-arginine methyl-ester (L-NAME), for 28 consecutive days [35]. The rats were randomized into different groups and treated as described below: Group 1 (Baseline/normal control) – took distilled water alone; Group 2 (Negative control/hypertensive control) – induced with L-NAME (40 mg/kg/day); Group 3 (Positive control/reference group) – induced with L-NAME, and given lisinopril (10 mg/kg); and Groups 4, 5, and 6 (Test groups) – induced with L-NAME, and administered with extracts from the dried calyx of *H. sabdariffa*, at varying doses of 250, 500, and 1000 mg/kg/day, respectively, and simultaneously. Lisinopril, and the extracts (from the calyx that were dried at different temperatures) were also administered orally to the rats for 28 consecutive days. The design and description are as illustrated in Table 1 below;.

2.5. Measurement of blood pressure, collection of samples, and assay determinations

About 24 h after the last treatment, the mean arterial blood pressures, as well as the diastolic and systolic pressures of the rats were taken

Table 1
Description of the experimental design.

Groups	Distilled Water	L-NAME (40 mg/kg)	Lisinopril (10 mg/kg)	*H. sabdariffa* extract (from calyx dried at different temperatures)			
				-58 °C	30 °C	40 °C	50 °C
Grp 1: BC	√	–	–	–	–	–	–
Grp 2: NC	–	√	–	–	–	–	–
Grp 3: PC	–		√	–	–	–	–
Grp 4: (250 mg/kg)	–	√	–	√	√	√	√
Grp 5: (500 mg/kg)	–	√	–	√	√	√	√
Grp 6: (1000 mg/kg)	–	√	–	√	√	√	√

Note: BC – Baseline Control; NC – Negative Control; PC – Positive Control; '√' – Orally administered; '-' – Not administered. N = 5 rats per group.

by utilizing the tail-cuff plethysmography process. An electro sphygmomanometer (CODA, Kent Scientific, USA) was used to take the readings. The average values of five consecutive determinations were taken and recorded as the pressure readings from each rat [35]. Afterwards, the final weights of the rats were recorded. They were subsequently subjected to an overnight fast, after which they were euthanized with isoflurane inhalation. They were then sacrificed by cervical dislocation, dissected, and their blood taken after a cardiac puncture with the use of a sterile syringe. The samples of the collected blood were stored inside ethylenediamine triacetate (EDTA) tubes. To obtain clear sera from the blood samples, the sample tubes were centrifuged at 3000 rpm, for a quarter of an hour. The clear sera were carefully aspirated into new plain sterile sample bottles and were stored at − 20 °C. All the required biochemical assays were done within a few days.

The sera were evaluated for the level of aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, alkaline phosphatase (ALP) activity, and gammaglutamyl transferase (GGT) activity, using a Selectra pros machine. Serum lipid profile (triglycerides, HDL- and LDL-cholesterol, total cholesterol), glucose, were evaluated with commercially available test kits from Randox (Randox Laboratories, England). The hearts and kidneys of the rats were also harvested, weighted and observed for gross lesions. They were subsequently homogenized and the homogenates used for the evaluation of oxidative stress/antioxidants. Glutathione peroxidase activity (GPx), catalase activity (CAT), superoxide activity (SOD), as well as lipid peroxidation (LPO or MDA) were evaluated to ascertain the presence or absence of oxidative stress. These antioxidant activities were evaluated in-line with the methods and protocols described in our previous studies [36–41].

2.6. . Measurement of the activities of angiotensin-1-converting enzyme (ACE), and arginase, as well as the levels of hydrogen peroxide (H_2O_2), and nitric oxide (NO)

The activity of ACE of the kidney tissues was measured following the protocol reported by Cushman and Cheung [42], where they enzymatically measured the level of hippuric acid that was severed from hippuryl-histidyl-leucine. The serum H_2O_2 concentration of the test rats (administered with L-NAME) were determined using PTO [$K_2TiO(C_2O_4)_2{\cdot}H_2O$] and spectrometric analysis at a wavelength of 400 nm [43]. A similar procedure was conducted on the extracts of *H. sabdariffa,* and the H_2O_2 generated were calculated from the calibration curve. The activity of arginase in the cortex of the kidney was measured by determining the rate at which urea is produced, using 9 % of α-iso--nitrosopropiophenone in absolute ethanol, and following the protocol that was earlier reported by Zhang et al. [44], where the arginase activity index was determined by measuring the amount of produced urea, after the normalization with protein. The amount of NO in the serum was evaluated in a solution which contains 400 mL of vanadium chloride (VCl_3) (2 %) in 5 % HCl, 200 mL of N-(L-naphthyl) ethylenediamine dihydrochloride (0.1 %), and 200 mL of sulfanilamide (2 %) in 5 % HCl, based on previously described protocol [45].

2.7. Statistical analysis

After the determination of the various parameters and their analyzes, the data that were acquired were presented as Means ± SEM. The data were also analyzed using one-way analysis of variance (ANOVA). Afterwards, they were subjected to Turkey multiple comparison tests. The criterion for the data been significant, statistically, was taken at a probability level of $p < 0.05$.

3. Results

3.1. Administration of the extracts from the dried calyx of H. sabdariffa resulted in decreases in total cholesterol, triglyceride, as well as LDL-cholesterol, but increases the HDL-cholesterol levels in normal experimental rats

Our results indicate the effects of administration of varying doses of the different extracts (with respect to the varying temperature of drying of the calyx) of *H. sabdariffa* on the lipid profile of the normal experimental rats, after 28 days (Fig. 1). In Fig. 1A, the amount of total cholesterol of the test rats exhibited a dose-dependent significant ($p < 0.05$) decrease, after 28 days of treatment with the different extracts. Comparatively, the decreasing effect was highest in the 30 °C-dried-calyx extract-treated group, irrespective of the administered dose. This trend was also observed in Fig. 1B, where the triglyceride level decreased with increasing dose of extract administration. However, at 1000 mg/kg dose of administration, the − 58 °C-dried-calyx extract-treated group recorded the least triglyceride level. In Fig. 1C, we show that treatment with the different extracts, in normal experimental rats, caused significant ($p < 0.05$) increases in the amount of serum HDL-cholesterol in all groups at an administration dose of 500 mg/kg, as well as at a dose of 250 mg/kg in the − 58 °C-, and 30 °C-dried-calyx extract-treated groups. But, at a dose of 1000 mg/kg, the HDL-cholesterol level of the test group did not significantly differ from that of the control. In contrast, the amount of LDL-cholesterol of the treated groups decreased significantly ($p < 0.05$), irrespective of the temperature at which the calyx was dried or the administered dose. However, comparatively, the decrease was in the order 30 °C-dried-calyx extract > − 58 °C-dried-calyx extract > 40 °C-dried-calyx extract > 50 °C-dried-calyx extract (Fig. 1D).

3.2. L-NAME resulted in increments of the total cholesterol, triglyceride, LDL, as well as decrement in HDL, while treatment with extracts from the dried calyx of H. sabdariffa reversed the trend in the hypertensive test rats administered with L-NAME

After observing different effects associated with the administration of the different extracts from *H. sabdariffa* calyx (after drying at different temperatures) on the profile of lipids in the normal experimental rats, we further investigated these effects in the hypertensive test rats administered with L-NAME (Fig. 2). Our results show that induction with L-NAME significantly ($p < 0.05$) increased the levels of total cholesterol, triglyceride, LDL, and significantly ($p < 0.05$) decreased the

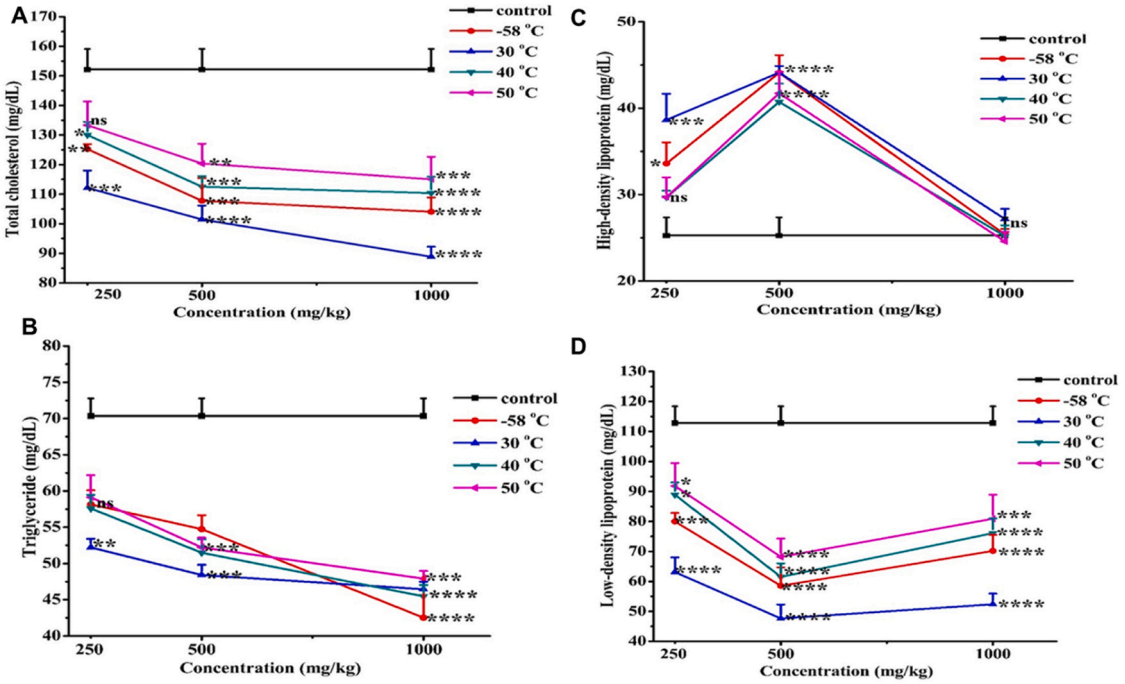

Fig. 1. Lipid profile. (A) Total cholesterol, (B) Triglyceride, (C) High-density Lipoprotein (HDL), (D) Low-density lipoprotein (LDL), of the experimental rats, after oral administration of different doses of extracts from the dried calyx of *H. sabdariffa,* for 28 days. Data are indicated as Means ± SEM, with n = 5. Data identified as *, **, ***, **** differ significantly at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$ respectively, while data identified with superscript "ns" do not differ significantly.

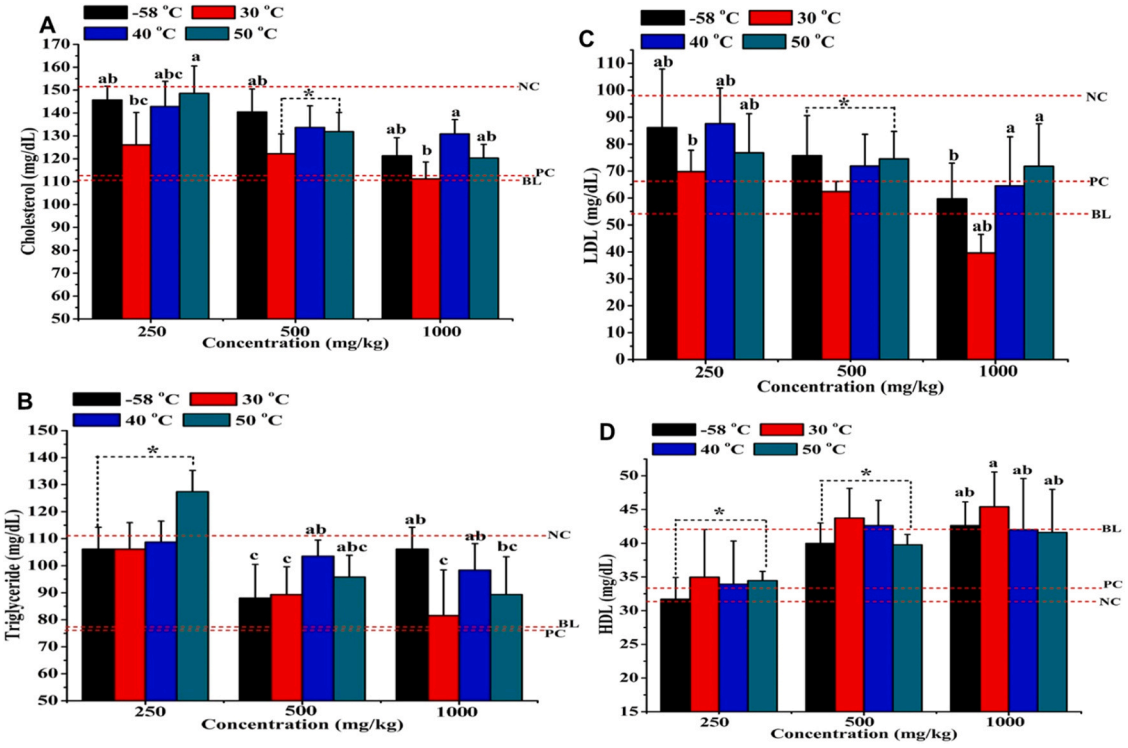

Fig. 2. Lipid profile. (A) Total cholesterol, (B) Triglyceride, (C) Low-density lipoprotein (LDL), (D) High-density lipoprotein (HDL), of the rats administered with L-NAME, after oral administration of different doses of extracts from the dried calyx of *H. sabdariffa,* for 28 days. BL – Baseline control; NC – Negative control; PC – positive control. Data are indicated as Means ± SEM, with n = 5. Data identified as *, [a], [b], [c] differ significantly. The letters a, b, c, as well as the symbol * indicate statistical significance or otherwise between and among the groups. The groups (represented by the different bars) with different letters are statistically different. While the groups with the same letter are not statistically different from each other.

HDL level in all the test groups (Fig. 2). However, in Fig. 2A, the administration of the extracts caused significant ($p < 0.05$) dose-dependent decreases of total cholesterol in the groups administered with L-NAME, in comparison with that of the group which served as the negative control. Among treated groups, the group administered with the 30 °C-dried-calyx extract had the lowest total cholesterol level, which is comparable to that of the group which served as the positive control, and was administered with lisinopril, at 1000 mg/kg of the administered dose. In contrast, the outcome of the extracts as it affects the test rats' triglyceride level were not dose-dependent (Fig. 2B). Generally, the triglyceride level of the test rats decreased, across the groups, on treatment with the extracts. On administration of 250 mg/kg dose, the effect of the − 58 °C-dried-calyx extract on the triglyceride level was higher, in a statistically significant ($p < 0.05$) manner, than the effect of the extract from the 50 °C-dried-calyx, but did not differ significantly from the effects of the 30 °C-dried-calyx extract and 40 °C-dried-calyx extract. But at 500 mg/kg dose, the − 58 °C-dried-calyx extract was reported to be more effective, while at 1000 mg/kg dose, the 30 °C-dried-calyx extract was more effective in lowering the triglyceride level of the test rats. Also, we recorded a dose-dependent decrease in the LDL level of the L-NAME-administered rats, as against that of the negative control group, after extract administration (Fig. 2C). Interestingly, at all the administered dose, the extract from the 30 °C-dried-calyx exhibited a higher reducing effect on the LDL level of the test rats. Thus, the 30 °C-dried-calyx extract exhibited the lowest LDL level, and was comparatively lower, in a significant manner, than it was in the baseline control when administered at a concentration of 1000 mg/kg. In contrast, we also recorded a dose-dependent increase in the HDL level of the L-NAME-administered rats, in contrast to that of the negative control, after extract administration (Fig. 2D). Interestingly, the HDL level of the *H. sabdariffa* extracts-treated groups were comparatively higher, in a significant ($p < 0.05$) manner, than the level of HDL of the positive control. Comparatively, the administration of the extract of the 30 °C-dried-calyx resulted in the highest HDL level.

3.3. Administration of extracts from the dried calyx of H. sabdariffa caused increases in antioxidant activities of cardiac tissues in normal rats

Our results indicate the outcome of the 28-day administration of different doses of extracts from the dried calyx of *H. sabdariffa* on the antioxidant enzyme activities of the cardiac tissues of the normal experimental rats (Fig. 3). Fig. 3A indicates that the GPx activity exhibited a dose-dependent increment, which is significant ($p < 0.05$) across all test groups. Comparatively, the increase is in the order 30 °C-dried-calyx extract > − 58 °C-dried-calyx extract > 40 °C-dried-calyx extract > 50 °C-dried-calyx extract. Although not significant and dose-dependent, the MDA/LPO level decreased across the test groups following treatment with the different extracts (Fig. 3B). But, catalase activity from the cardiac tissues of the test rats rose in a significant ($p < 0.05$) manner after administration of the different doses (250, 500, and 1000 mg/kg) of the extracts (Fig. 3C). Comparing the different groups treated with the extracts, the groups administered with the 40 °C-dried-calyx extract, and the 30 °C-dried-calyx extract exhibited increases in the activity of SOD in a significant ($p < 0.05$) fashion (Fig. 3D). Comparatively, the 40 °C-dried-calyx extract tend to be more effective. Overall, the resulting outcome of treatment with the extracts on the activity of SOD of the test rats decreases in the order 40 °C-dried-calyx extract > 30 °C-dried-calyx extract > 50 °C-dried-calyx extract > − 58 °C-dried-calyx extract.

3.4. Administration of extracts from the dried calyx of H. sabdariffa caused increases in antioxidant activities of renal tissues in normal experimental rats

Fig. 4 show the effect of the 28-day administration of different doses of extracts from the dried calyx of *H. sabdariffa* on activities of antioxidant enzyme of the renal tissues of the normal experimental rats. Our findings show that GPx activity of renal tissues significantly ($p < 0.05$) and dose-dependently increased, after extract administration (Fig. 4A).

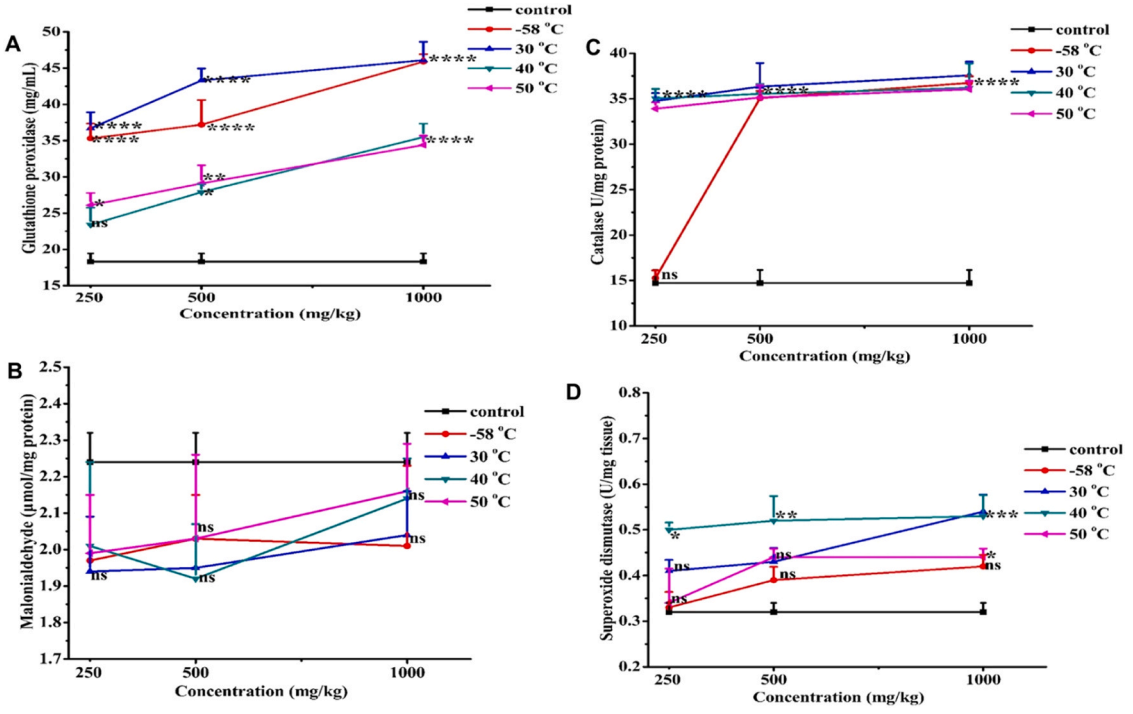

Fig. 3. Antioxidant enzymatic activity [A – Glutathione peroxidase (GPx); B – Malondialdehyde/Lipid peroxidation (MDA/LPO); C – Catalase (CAT); D – Superoxide dismutase (SOD)] of the cardiac tissue of the experimental rats, after oral administration of different doses of the extracts from the dried calyx of *H. sabdariffa,* for 28 days. Data are indicated as Means ± SEM, with n = 5. Data identified as *, **, ***, **** differ significantly at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$ respectively, while data identified with superscript "ns" do not differ significantly.

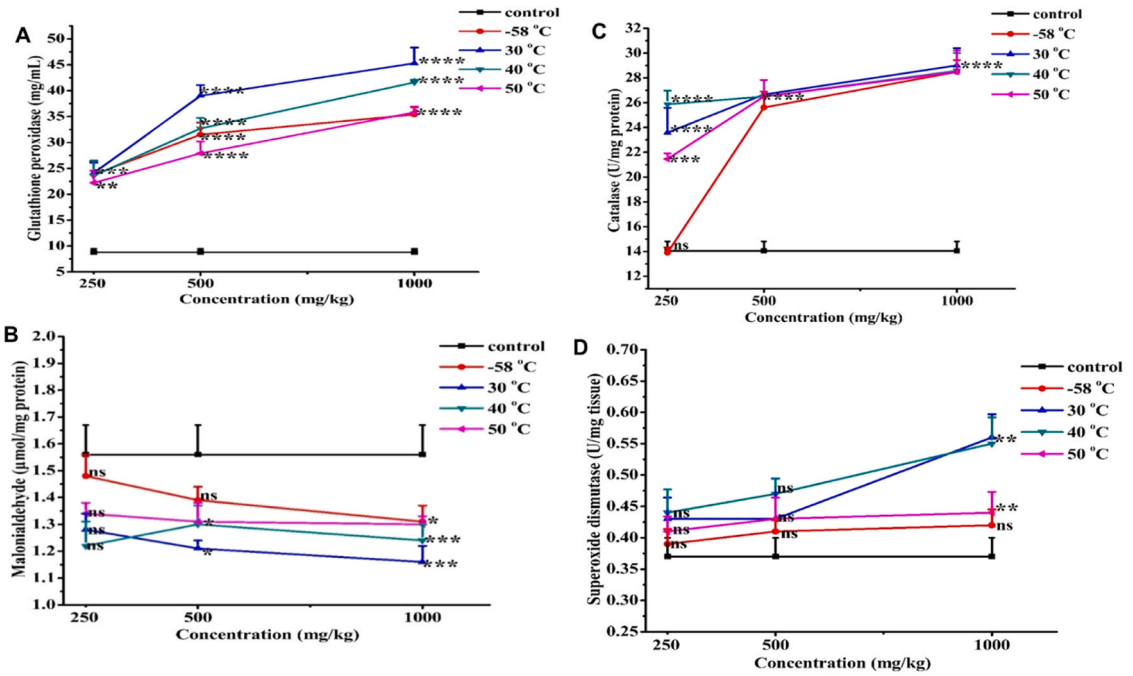

Fig. 4. Antioxidant enzymatic activity [A – Glutathione peroxidase (GPx); B – Malondialdehyde/Lipid peroxidation (MDA/LPO); C – Catalase (CAT); D – Superoxide dismutase (SOD)] of the renal tissue of the experimental rats, after oral administration of different doses of the extracts from the dried calyx of *H. sabdariffa,* for 28 days. Data are indicated as Means ± SEM, with n = 5. Data identified as *, **, ***, **** differ significantly at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$ respectively, while data identified with superscript "ns" do not differ significantly.

In addition, it was observed that the highest range of GPx level was recorded in the test group administered with the 30 °C-dried-calyx extract. Equally worthy of note is the effects of the extract on the activity of GPx of the renal tissues which decreases in the order 30 °C-dried-calyx extract > 40 °C-dried-calyx extract > − 58 °C-dried-calyx extract > 50 °C-dried-calyx extract. Apart from the test group administered with the − 58 °C-dried-calyx extract, administration of the other extracts to their respective test groups caused significant ($p < 0.05$) reductions in the level of their MDA/LPO, at 500 mg/kg dose of administration. But, at a dose of 1000 mg/kg, the level of MDA/LPO of every test group, administered with different extracts, decreased significantly ($p < 0.05$), in contrast to that obtained for the control group. Comparatively, the 30 °C-dried-calyx extract tend to be more effective (Fig. 4B). Also, in Fig. 4C, the activity of catalase for the renal tissues from the test rats increased. In comparison to that of the rats in the control group, the increases were significant ($p < 0.05$), and directly proportional to increase in dose of the administered extract. Comparatively, the 30 °C-dried-calyx extract was also shown to be more effective. At extract doses of 250 mg/kg, and 500 mg/kg, SOD activity increased, non-significantly, in the test groups, in contrast with the control group, irrespective of the administered extract. Nevertheless, at a dose of 1000 mg/kg, the 30 °C-dried-calyx, 40 °C-dried-calyx, and 50 °C-dried-calyx extracts caused the activity of renal SOD of the test rats to increase significantly ($p < 0.05$), in comparison to that of the rats in the control group.

3.5. Induction with L-NAME led to increased MDA/LPO and H_2O_2 levels, and decreased GPx and SOD activities, while administration of extracts from the dried calyx of H. sabdariffa caused reversal of these effects in the L-NAME-administered test rats

Due to increasing effects of administration of the extracts from the dried calyx of *H. sabdariffa* on antioxidant levels in the normal experimental rats, we further investigated these effects in the hypertensive test rats administered with L-NAME. Our results (Fig. 5) show that when L-NAME was administered to the test rats, it resulted in significant ($p < 0.05$) increases in MDA/LPO level, as indicated in Fig. 5A, and H_2O_2, as indicated in Fig. 5B (an indication of lipid peroxidation and manifestation of oxidative stress), in comparison with that of the control. L-NAME also caused reductions ($p < 0.05$) in GPx activity (Fig. 5C), and SOD activity (Fig. 5D) of the rats in the test groups, as against those of the control group. Nevertheless, administration of the extracts from the dried calyx of *H. sabdariffa* caused a reversal of these effects in the hypertensive test rats administered with L-NAME. Administration of varying doses of the extracts resulted in significant reduction in MDA/LPO of the test groups, and these effects favorably competes with that of the control drug, lisinopril (positive control) (Fig. 5A). Comparatively, the 30 °C-dried-calyx extract was more effective at the 250 mg/kg dose, while the 50 °C-dried-calyx extract was more effective at the 500 mg/kg dose, and the 40 °C-dried-calyx extract was more effective at the 1000 mg/kg dose of administration. Similarly in Fig. 5B, treatment of the L-NAME-administered hypertensive rats with the extracts caused a significant reduction of the generated H_2O_2 in the test rats, and the reduction was same as that obtained for the control drug (lisinopril). Generally, at 250 mg/kg dose of administration, the extracts exhibited better effect. However, the extract from the 30 °C-dried-calyx was comparatively more effective at the doses of 250 mg/kg, and 500 mg/kg, while the extract from the 50 °C-dried-calyx was more effective at the 1000 mg/kg administered dose. In Fig. 5C, post administration with the control drug (Lisinopril) resulted in significantly increased level of GPx in L-NAME-administered hypertensive albino rat model. Among test groups, no significant differences were observed in glutathione peroxidase activity at the lowest and highest doses (250 mg/kg, and 1000 mg/kg, respectively), in comparison with the control. However, treatment with the extracts at 500 mg/kg dose in the L-NAME-administered hypertensive albino rat model resulted in significantly increased GPx level

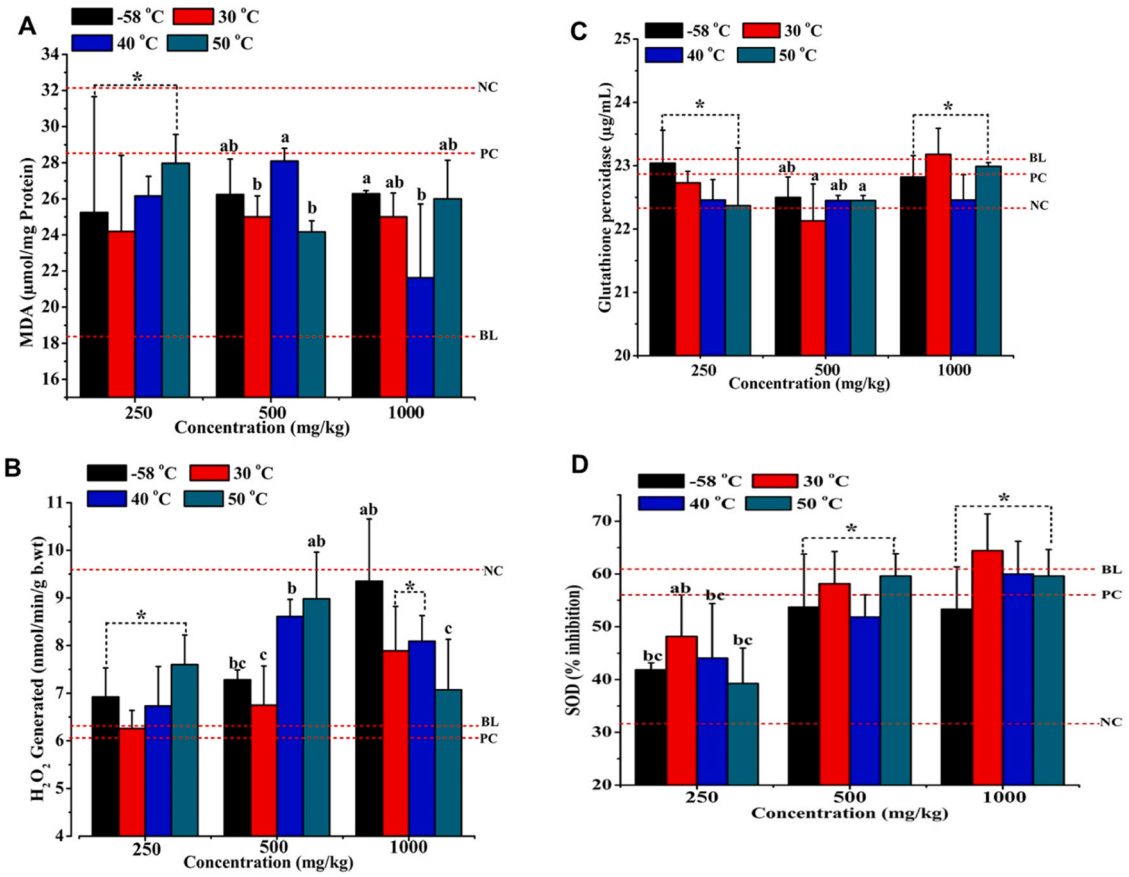

Fig. 5. Antioxidant enzymatic activity [A – Malondialdehyde/Lipid peroxidation (MDA/LPO); B – Glutathione peroxidase (GPx); C – Catalase (CAT); D – Superoxide dismutase (SOD)] of the rats administered with L-NAME, after oral administration of different doses of extracts from the dried calyx of *H. sabdariffa,* for 28 days. BL – Baseline control; NC – Negative control; PC – positive control. Data are indicated as Means ± SEM, with n = 5. Data identified as *, ^{a}, ^{b}, ^{c} differ significantly. The letters a, b, c, as well as the symbol * indicate statistical significance or otherwise between and among the groups. The groups (represented by the different bars) with different letters are statistically different. While the groups with the same letter are not statistically different from each other.

in a similar fashion as observed in the control group. Interestingly, the − 58°C-dried-calyx extract (at a dose of 250 mg/kg) and the 30 °C-dried-calyx extract (at 1000 mg/kg dose) were comparatively more effective. Also, in Fig. 5D, post–treatment with the positive control drug (lisinopril) resulted in significantly ($p < 0.05$) higher activity of SOD in the test groups, while the different extracts caused a dose-dependent, and significant ($p < 0.05$) increment in SOD activity from the test group, as against that of the negative control rats. Interestingly, the 30 °C-dried-calyx extract proved to be the most effective, at all the administered doses.

3.6. *Induction with L-NAME led to increased diastolic and systolic pressures, while administration of extracts from the dried calyx of H. sabdariffa caused reductions in these pressures in the L-NAME-administered test rats*

Fig. 6 show that induction with L-NAME in the experimental rats resulted in significant ($p < 0.05$) increases in systolic, and diastolic pressures of the test rats. A rise in the blood pressures (systolic and diastolic), which was significant ($p < 0.05$), in the experimental animals was observed in the negative control. Oral administration of extracts from the dried calyx of *H. sabdariffa* for 28 days brought about significant reduction in the systolic and diastolic blood pressure in the experimental animals, and the effects were comparable to that of the baseline control. Apart from the effect on the diastolic pressure at the 250 mg/kg dose of extract administration, the 30 °C-dried-calyx extract was more effective at decreasing the systolic and diastolic pressure in the L-NAME-administered test rats.

3.7. *The induction with L-NAME caused significant increases in ACE and arginase activities, glucose concentration, and significant reduction in nitric oxide activity, while administration of extracts from the dried calyx of H. sabdariffa reversed these effects in the L-NAME-administered test rats*

After confirming the systolic and diastolic pressure reducing effects of the different extracts in the L-NAME-administered test rats, we further determined the effects of the extracts on other biomarkers of hypertension in the experimental rats. Induction with L-NAME resulted in significant ($p < 0.05$) increases in ACE and arginase activities, as well as the level of glucose (Fig. 7). The induction also brought about significant ($p < 0.05$) decrease in nitric oxide activity in the experimental rats. However, administration of the different extracts caused a significantly ($p < 0.05$) reduced activity of ACE of the experimental rats, as compared with that of the baseline control group (Fig. 7A). The effect could be likened to that observed in the positive control, administered with lisinopril. Interestingly, only the extract from the calyx, dried at − 58 °C (250 mg/kg administered dose), 30 °C (500 mg/kg dose), and 50 °C (at 1000 mg/kg dose), were able to decrease the activity of ACE to an amount lower than that from the positive control group. The arginase activity was also reduced after the administration of the different extracts (Fig. 7B). The decrease in the arginase activity of the test rats was dose-dependent, thus, as the dose of administration increased, the arginase activity decreased. Overall, treatment with varying doses of the extracts significantly decreased ($p < 0.05$) the activity of arginase in the experimental group to a relatively lower level than that observed in the negative control group. Also, nitric oxide level was changed after extract

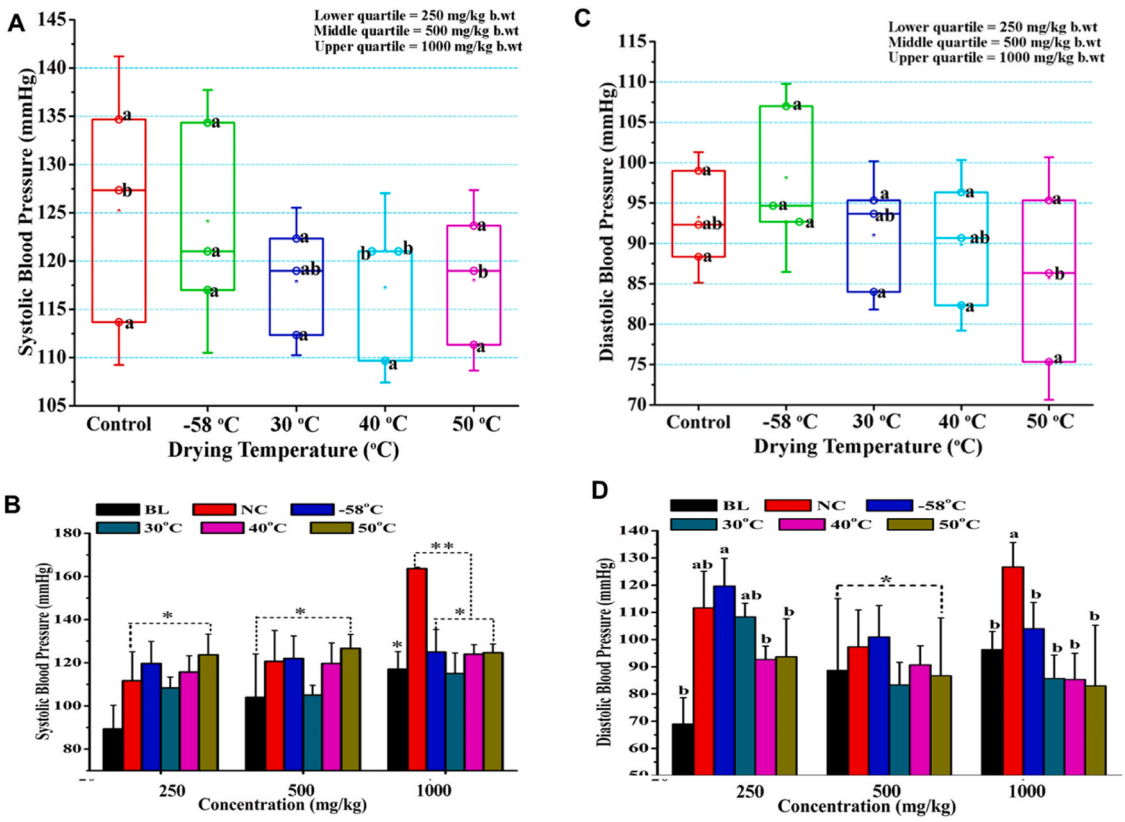

Fig. 6. Changes in blood pressure of the L-NAME-administered test rats., after oral administration of different doses of extracts from the dried calyx of *H. sabdariffa,* for 28 days. (A&B) systolic pressure; (C&D) diastolic pressure. BL – Baseline control; NC – Negative control. Data are indicated as Means ± SEM, with n = 5. Data identified as *, **, [a], [b], differ significantly.

administration to the L-NAME-administered test rats (Fig. 7C). Administration of varying doses of the extracts, caused significantly ($p < 0.05$) higher nitric oxide amount in the test rats. However, the 40 °C-dried-calyx extract (250 mg/kg and 500 mg/kg) and − 58 °C-dried-calyx extract (at 1000 mg/kg dose) were comparatively more effective. In Fig. 7D, the glucose level of the test rats decreased significantly ($p < 0.05$) than as observed in the negative control rats, and in comparison, with that of the baseline control rats. Also, the 40 °C-dried-calyx extract (250 mg/kg and 500 mg/kg), and 50 °C-dried-calyx extract (at 1000 mg/kg dose) were comparatively more effective at lowering the level of glucose in the L-NAME-administered test rats.

4. Discussion

Prior to its use, the drying temperature of *H. sabdariffa* calyx possibly affects the bioavailability or potency of its phytocomponents. In our previous report, it was stated that *H. sabdariffa* extract show different types and quantities of phytochemicals, with respect to the drying temperature of the calyx. We reported the absence of steroids, and coumarin glycosides at 50 °C drying temperature, while healthy levels of saponin, alkaloids, tannin, phenols, and flavonoids, were recorded after drying at 30 °C [33]. Alkaloids have been well implicated in the traditional treatment of hypertension [1,37], anthocyanins and saponins are known for their anti-lipidemic activities, while the cytotoxic, antioxidant, antiviral, and anti-inflammatory activities of flavonoids are popular, and recently, as promising medicinal candidates with anti-hypertensive and anti-diabetic effects [30,46,47]. Thus, these phytochemicals have been implicated in the ethnomedical treatment of some diseases like hyperlipidaemia, oxidative stress-related and cardiovascular diseases [7,28,40,41].

Findings from our present study show that the administration of *H. sabdariffa* extracts to the experimental rats (whether administered with L-NAME or not), was able to bring about decreases in their serum triglycerides, LDL-cholesterol, and total-cholesterol. This treatment, which also caused a healthy rise in the level of HDL-cholesterol, indicate the hypolipidemic benefits of *H. sabdariffa*. This agrees with previous studies which reported the lipid-lowering activity of *H. sabdariffa* extracts, which is vital in the prevention of diseases such as hyperlipidaemia, and other cardiovascular-related diseases [48–50]. Blood lipids like total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol provide vital information on the predisposition of some vital organs to abnormal lipid metabolism as well as other cardiovascular disorders. Abnormal or high levels of lipids often results in hyperlipidaemia, hypercholesterolemia, hypertension, and uncontrolled diabetes [26,51,52]. The lipid-lowering property of *H. sabdariffa* in the rats administered with L-NAME shows its potential as an antihypertensive agent, which can find a good place in the management regimen for cardiovascular-related diseases. This is in agreement with the study carried out by Donfack's et al. [53], where they also reported the lipid-lowering properties of the stem-bark extract of *Vitex cienkowskii* in L-NAME-induced hypertensive rats. Interestingly, this property could also be affected by the temperature at which the calyx was dried. This is because our findings show that the drying temperature affects the effectiveness of the extract. Here, we report that the lipid-lowering property of *H. sabdariffa* extract decreases in the order 30 °C-dried-calyx extract > − 58 °C-dried-calyx extract > 40 °C-dried-calyx extract > 50 °C-dried-calyx extract.

Apart from *H. sabdariffa's* ability to cause decreases in serum lipid, findings from our present work also indicate that treatment with extracts from the calyx, in the normal experimental rats, was able to bring about increases in the antioxidant activities in some key tissues in these rats. Specifically, the cardiac and renal tissues were shown to exhibit high amounts of antioxidant activities, which could pass as an ability of *H. sabdariffa* to defend these tissues against oxidative stress. It is worthy of

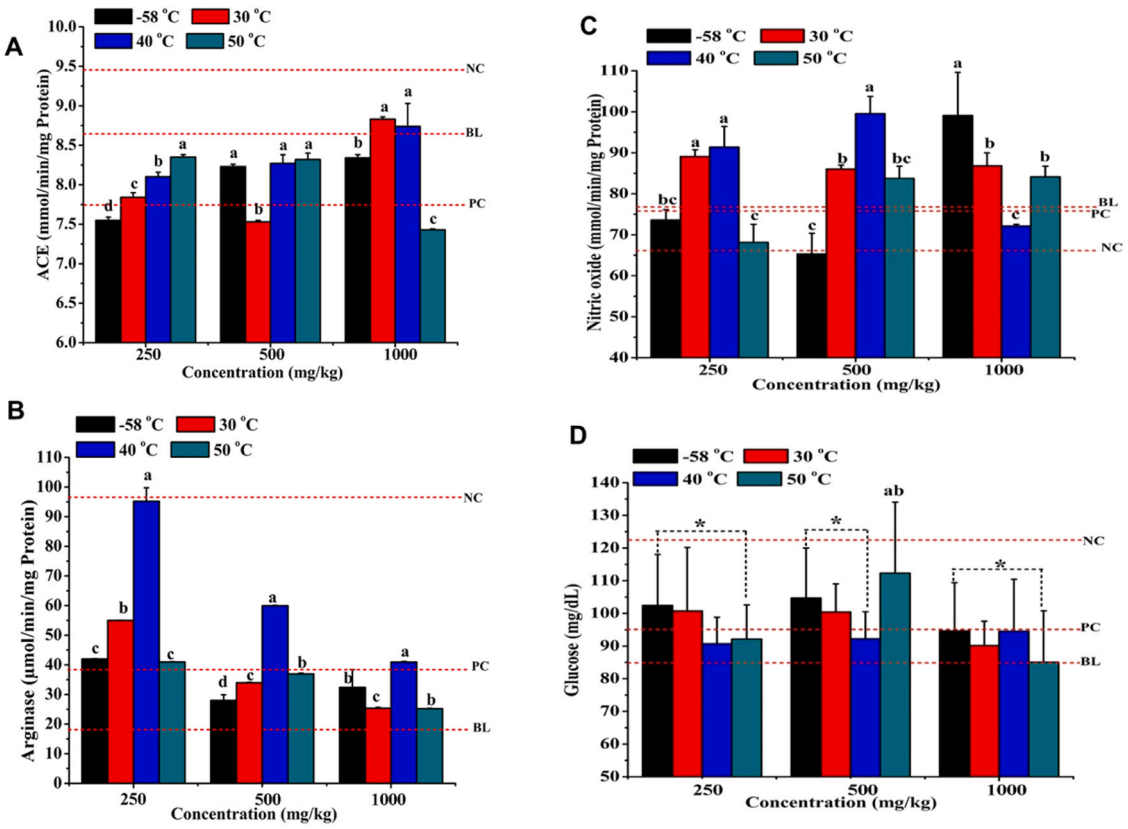

Fig. 7. Activities of biomarkers of hypertension [A – Angiotensin I-converting enzyme (ACE); B – Nitric Oxide; C – Arginase; D – Glucose] of the L-NAME-administered test rats., after oral administration of different doses of extracts from the dried calyx of *H. sabdariffa,* for 28 days. BL – Baseline control; NC – Negative control; PC – positive control. Data are indicated as Means ± SEM, with n = 5. Data identified as *, ^{a}, ^{b},^{c}, differ significantly. The letters a, b, c, as well as the symbol * indicate statistical significance or otherwise between and among the groups. The groups (represented by the different bars) with different letters are statistically different. While the groups with the same letter are not statistically different from each other.

note that the 30 °C-dried-calyx extract was comparatively the most effective in this respect. Previously, Gauthaman and colleagues, [54] reported how *Hibiscus rosasinensis* flower augmented endogenous antioxidant compounds in the heart of experimental rat, and prevented isoproterenol-induced myocardial injury in the myocardium. Just as Khattab et al. [55] reported the attenuation of Adriamycin-induced cardiotoxicity in experimental rats by *H. sabdariffa* through the bioactivities of its antioxidant's constituents. While Banerjee et al. [56], infer that the mechanism of protection may be attributed to myocardial adaptation of the rats, since oxidative stress is resolved by the support from cellular antioxidants like SOD, glutathione, catalase and the nutraceutical phytochemicals inherent in the experimental plant such as flavonoids, glycosides, and tannins. The rise in the activities of antioxidant in the rats, which is related to the increase in the cellular antioxidants as described in the result section, points at the possible mechanism by which the calyx extract of *H. sabdariffa* protects these tissues from oxidative stress. For example, this may help in protecting the cardiac tissues from developing some forms of cardiovascular diseases, which is usually associated with the production of free radicals, and the building-up of oxidative stress. The kidney's capacity to ensure optimal condition of body fluids may also be affected by the production of free radicals, with consequent oxidative stress condition. Usually, an increase in the antioxidant defense system, as indicated by the effects of the administered extracts, has the potential to protect renal tissues against the onslaught of free radicals. This, in turn, prevents any form of oxidative stress-related disease or damage to the renal tissues.

The potential of *H. sabdariffa* to protect against oxidative stress-related diseases or cardiovascular diseases, like hypertension, as evaluated in the L-NAME-administered rats, competes favorably with that of the standard drug, lisinopril. The antioxidant enzymes' activities, or that of the oxidative-stress biomarkers, were significantly increased after treatment of the L-NAME-administered hypertensive rats with the different extracts. The prevention or slowing down of lipid peroxidation (MDA) in the hypertensive rats, an effect resulting from the treatment with the extracts from the dried calyx (at varying temperature) of *H. sabdariffa*, points at the defensive capacity of the plant against unwanted oxidation of the lipids of the cell membranes. This helps to maintain the integrity of the cell membranes and proper tissue function. Our result is in agreement with the report from the work done by Godwill [57] which stated that extracts from *H. sabdariffa* act as antioxidants that react preferentially to inactivate ROS and enhance cellular antioxidant defenses for the protection of cells from free radical damages. Interestingly, and in line with this, we observed that the 30 °C-dried-calyx extract show higher antioxidative and antihypertensive potential.

Several studies have validated the fact that L-NAME, when chronically administered to rats, induces arterial hypertension which is related with nitric oxide (NO) insufficiency [22,53,58,59]. This was corroborated by our results which show increases in the arterial blood pressure of the experimental rats after L-NAME administration. This effect could be attributed to L-NAME's ability to block the generation of the popular vasodilator molecule, nitric oxide, through the inhibition of nitric oxide synthase (NOS) activity. However, the administration of *H. sabdariffa* calyx extracts were able to bring about decreases in the arterial blood pressures (both systolic and diastolic) of the hypertensive test rats. The effect, which indicates the antihypertensive property of the plant, also favorably compares with that of the control drug (lisinopril). For the regulation of hypertension, the renin-angiotensin system (RAS) is known to perform major, and specific functions in its pathogenesis and

advancement [16]. Hence, one of the pharmacological bases of its regulation is through the inhibition of the activity of the angiotensin I-converting enzyme (ACE). Other pharmacological basis of its regulation is through the inhibition of the upstream activity of renin, or that of the receptors of downstream angiotensin [16]. The significant increases in the activities of ACE, and arginase, as well as glucose level, and a significant reduction in the activity of nitric oxide, which occurred after the administration of L-NAME, were reversed after administration with the different extracts from the calyx of *H. sabdariffa*. Apart from its hypoglycemic effect, the decrease in ACE or inhibition of ACE, as occasioned by the administration of the extracts, points at the therapeutic potential of the plant. As an alternative to drugs, the inhibition of ACE is the major target; especially developing by food-originated therapies [60]. *H. sabdariffa* could possibly pass for this since its antihypertensive activities involve the inhibition of ACE. This effect could also be associated with the combined effects of the phenol-derived compounds present in *H. sabdariffa*, as we reported in our previous study [33]. Phenolic compounds have earlier been shown to block the activity of ACE, either individually or collectively with some other related compounds [61]. In line with our present study, other works have reported that L-NAME, when administered orally, caused remarkable increase in the arterial blood pressure, due to the prevention of NO synthesis, as well as the continuous promotion of the renin–angiotensin system [62–64]. Nitric oxide functions as an effective vasodilator, and performs vital roles in the maintenance of the tone of the vasculature [65]. NO is primarily generated in the kidney by neuronal NOS, and nitric oxide synthase (eNOS) [66]. Although arginine, which usually participate in several biochemical pathways, is principally perceived as a precursor for the synthesis of NO. However, treatment with *H. sabdariffa* extracts which caused a dose-dependent reduction in the arginase activity; as well as a concomitant rise in the level of NO in the L-NAME--administered rats, shows the possible role played by the extracts from the dried calyx (at varying temperature) of *H. sabdariffa* in vasodilation, maintenance of vascular tone, and decrease in arterial blood pressure, which is vital in managing hypertension, and other diseases of cardiovascular origin.

The limitation of this study is that we were not able to carry out detailed isolation and analysis of the individual phytoconstituents or nutraceuticals from the calyx of *H. sabdariffa,* as well as determine the possible mechanism of action of some of them. This would have formed the basis for the scientific rationale for its use in the management of cardiovascular diseases. However, further studies can focus on this aspect to give a possible scientific justification for the use of *H. sabdariffa* in ethnomedicine.

5. Conclusion

We report that *H. sabdariffa* calyx extract possesses hypolipidemic, antioxidant, and antihypertensive properties. The potency of the extracts, which varies with the drying temperature of the calyx, portends the potential of *H. sabdariffa* as a potent medicinal agent, which could be useful in treating hypertension, and other cardiovascular-related diseases. In support of these, more studies are recommended in view of its use in ethnomedicine.

Ethics approval and consent to participate

Ethical approval was secured from the Research Ethics Committee of the Department of Biochemistry, Ambrose Alli University, Ekpoma Nigeria. The use of rats was in accordance with the Ethical Guidelines Involving Whole Animal Testing of the Research Ethics Committee of the Department of Biochemistry, Ambrose Alli University, Ekpoma Nigeria, and the reporting in the manuscript follows the recommendations in the ARRIVE guidelines. Consent to participate is not applicable.

Consent for publication

N/A.

Funding

No funding was received for this research.

CRediT authorship contribution statement

John Osarenren Efosa, and Marshall Arebojie Azeke conceived, designed and performed the experiments; John Osarenren Efosa, Kingsley Omage, and Marshall Arebojie Azeke performed the analysis and interpretation of the data, while Kingsley Omage prepared the draft of the manuscript. All authors have reviewed and approved the final draft of the manuscript. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Availability of Data and Material

The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgement

We acknowledge the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria, for providing the space for the conduct of the experiments. The laboratory equipment grant awarded to Prof. Marshall A. Azeke by the Alexander von Humboldt Foundation Germany, as provided by the German Federal Ministry of Economic Cooperation and Development, is gratefully acknowledged.

Institutional Review Board Statement

The experimental procedure of this study was approved and carried out at the laboratory of the Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

References

[1] K. Omage, A.M. Azeke, O.S. Omage, Evaluation of the efficacy of *Acalypha wilkesiana* leaves in managing cardiovascular disease risk factors in rabbits exposed to salt-loaded diets, Clin. Phytosci. 4 (1) (2018) 1–7, https://doi.org/10.1186/s40816-018-0060-4.

[2] M.F. Yuyun, K. Sliwa, A.P. Kengne, A.O. Mocumbi, G. Bukhman, Cardiovascular diseases in sub-saharan Africa compared to high-income countries: an epidemiological perspective, Glob. Heart 15 (1) (2020) 15, https://doi.org/10.5334/gh.403.

[3] A.C. Montezano, R.M. Touyz, Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies, Ann. Med. 44 (1) (2012) S2–S16.

[4] A. Baradaran, H. Nasri, M. Rafieian-Kopaei, Oxidative stress and hypertension: possibility of hypertension therapy with antioxidants, J. Res. Med. Sci.: Off. J. Isfahan Univ. Med. Sci. 19 (4) (2014) 358–367. ⟨https://pubmed.ncbi.nlm.nih.gov/25097610⟩.

[5] G.Y.H. Lip, E. Edmunds, S.L. Nuttall, M.J. Landray, A.D. Blann, D.G. Beevers, Oxidative stress in malignant and non-malignant phase hypertension, J. Hum. Hypertens. 16 (5) (2002) 333–336.

[6] O.G. Erifeta, K. Omage, S.E. Uhunmwangho, K.H. Njoya, O.F. Amegor, C. A. Okonkwo, Comparative evaluation of antioxidant effects of watermelon and

orange, and their effects on some serum lipid profile of wistar albino rats, Int. J. Nutr. Metab. 3 (8) (2011) 97–102, https://doi.org/10.5897/IJNAM.9000006.
[7] K. Omage, O.G. Erifeta, S.E. Uhunmwangho, J.S. Josiah, O.K. Ajeigbe, Evaluation of hypoglycemic and antioxidative properties of aqueous extract of *Garcinia kola* seeds in wistar rats, Curr. Res. J. Biol. Sci. 3 (4) (2011) 326–329.
[8] O.S. Iseghohi, N.E.J. Orhue, K. Omage, Pre-exposure to *Dennettia tripetala* ethanolic fruit extract prevents biochemical alterations in rats subsequently exposed to a single dose of carbon tetrachloride, Int. J. Pharm. Phytochem. Ethnomed. 6 (2017) 8–16, https://doi.org/10.18052/www.scipress.com/IJPPE.6.8.
[9] K. Omage, O.S. Omage, Evaluation of the glycemic indices of three commonly eaten mixed meals in Okada, Edo State, Food Sci. Nutr. 6 (2018) 220–228, https://doi.org/10.1002/fsn3.550.
[10] U. Usunobun, J.S. Josiah, C.O.S. Nwangwu, S.E. Uhunmwangho, K. Omage, N. E. Maduagwu, Toxicity evaluation of the liver and in vitro metabolism in wistar rat on exposure to N-nitrosamine precursors, Brit. J. Pharm. Toxicol. 2 (3) (2011) 138–142.
[11] K. Omage, A.M. Azeke, N.E.J. Orhue, Implications of oral administration of extracts of *Acalypha wilkesiana* leave on serum electrolytes, urea and creatinine in normal experimental rabbits, Biokemistri 27 (2) (2015) 56–62.
[12] X. Dai, L. Hua, Y. Chen, J. Wang, J. Li, F. Wu, C. Liang, Mechanisms in hypertension and target organ damage: is the role of the thymus key? (Review), Int. J. Mol. Med. 42 (1) (2018) 3–12, https://doi.org/10.3892/ijmm.2018.3605.
[13] R. Dhalaria, R. Verma, D. Kumar, S. Puri, A. Tapwal, V. Kumar, K. Kuca, Bioactive compounds of edible fruits with their anti-aging properties: a comprehensive review to prolong human life, Antioxidants 9 (11) (2020), https://doi.org/10.3390/antiox9111123.
[14] L.C. Van-Vark, M. Bertrand, K.M. Akkerhuis, J.J. Brugts, K. Fox, J.J. Mourad, E. Boersma, Angiotensin-converting enzyme inhibitors reduce mortality in hypertension: a meta-analysis of randomized clinical trials of renin-angiotensin-aldosterone system inhibitors involving 158,998 patients, in: Q.-a. Reviews (Ed.), Database of Abstracts of Reviews of Effects (DARE): Quality-assessed Reviews, 2012. ⟨https://www.ncbi.nlm.nih.gov/books/NBK116055/⟩.
[15] C.C. Lau, N. Abdullah, A.S. Shuib, N. Aminudin, Proteomic analysis of antihypertensive proteins in edible mushrooms, J. Agric. Food Chem. 60 (2012) 12341–12348.
[16] A.J. Akinyemi, G.R. Thomé, V.M. Morsch, N.B. Bottari, J. Baldissarelli, L.S. de-Oliveira, M.R. Schetinger, Dietary supplementation of ginger and turmeric rhizomes modulates platelets ectonucleotidase and adenosine deaminase activities in normotensive and hypertensive rats, Phytother. Res. 30 (7) (2016) 1156–1163, https://doi.org/10.1002/ptr.5621.
[17] K. Omage, A.M. Azeke, *Acalypha wilkesiana* regulates fluid volume but affects selected tissues in salt loaded rabbits, Clin. Phytosci. 5 (10) (2019) 1–9, https://doi.org/10.1186/s40816-019-0103-5.
[18] E. Jiménez-Ferrer, J. Alarcón-Alonso, A. Aguilar-Rojas, A. Zamilpa, C.I. Jiménez-Ferrer, J. Tortoriello, M. Herrera-Ruiz, Diuretic effect of compounds from Hibiscus sabdariffa by modulation of the aldosterone activity, Planta Med. 78 (18) (2012) 1893–1898.
[19] M. Sarr, S. Ngom, M.O. Kane, A. Wele, D. Diop, B. Sarr, A.S. Diallo, *In vitro* vasorelaxation mechanisms of bioactive compounds extracted from *Hibiscus sabdariffa* on rat thoracic aorta, Nutr. Metab. 2 (6) (2009) 45–50.
[20] D. Ojeda, E. Jiménez-Ferrer, A. Zamilpa, A. Herrera-Arellano, J. Tortoriello, L. Alvarez, Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin-andcyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*, J. Ethnopharmacol. 8 (127) (2010) 7–10.
[21] A. Ahmad, S.K. Dempsey, Z. Daneva, M. Azam, N. Li, P.L. Li, J.K. Ritter, Role of nitric oxide in the cardiovascular and renal systems, Int. J. Mol. Sci. 19 (9) (2018), https://doi.org/10.3390/ijms19092605.
[22] A.M. Cardoso, L.H. Manfredi, D. Zanini, M.D. Bagatini, J.M. Gutierres, F. Carvalho, M.R.C. Schetinger, Physical exercise prevents memory impairment in an animal model of hypertension through modulation of CD39 and CD73 activities and A2A receptor expression, J. Hypertens. 37 (1) (2019). ⟨https://journals.lww.com/jhypertension/Fulltext/2019/01000/Physical_exercise_prevents_memory_impairment_in_an.22.aspx⟩.
[23] S. Wachtel-Galor, I.F.F. Benzie, An introduction to its history, usage, regulation, current trends, and research needs, in: Herbal Medicine, CRC Press/Taylor & Francis, Boca Raton (FL), 2011.
[24] A. Shaito, D.T.B. Thuan, H.T. Phu, T.H.D. Nguyen, H. Hasan, S. Halabi, G. Pintus, Herbal medicine for cardiovascular diseases: efficacy, mechanisms, and safety, Front. Pharm. 11 (2020) 422, https://doi.org/10.3389/fphar.2020.00422.
[25] O.S. Innih, I.G. Eze, K. Omage, Evaluation of the haematinic, antioxidant and anti-atherosclerotic potential of *Momordica charantia* in cholesterol-fed experimental rats, Toxicol. Rep. 9 (2022) 611–618, https://doi.org/10.1016/j.toxrep.2022.03.042.
[26] C.O.S. Nwangwu, J.S. Josiah, U. Usunobun, U. Nwangwu, A.A. Akintola, O. I. Ehiremen, K. Omage, Effects of aqueous and ethanolic extract of *Vernonia amygdalina* leaf on the plasma lipid profile and liver function parameters of normal rats, Curr. Res. J. Biol. Sci. 3 (5) (2011) 504–508.
[27] S.E. Uhunmwangho, K. Omage, O.G. Erifeta, J.S. Josiah, C.O.S. Nwangwu, Possible reversal of sodium arsenate-induced liver toxicity by hexane leaf extract of *Alchornea laxiflora*, Asian J. Med. Sci. 5 (1) (2013) 3–8, https://doi.org/10.19026/ajms.5.5339.
[28] O.S. Omage, N.E.J. Orhue, K. Omage, Evaluation of the phytochemical content, in vitro antioxidant capacity, biochemical and histological effects of *Dennettia tripetala* fruits in healthy rats, Food Sci. Nutr. 7 (2019) 65–75, https://doi.org/10.1002/fsn3.792.
[29] O.S. Omage, N.E.J. Orhue, K. Omage, *Dennettia tripetala* relieves chronic hepatorenal injuries in rats by altering fas, sod-1, and tnf-α expression, Prev. Nutr. Food Sci.. 27 (1) (2022) 89–98, https://doi.org/10.3746/pnf.2022.27.1.1 (ISSN 2287-8602).
[30] D.R. Pant, N.D. Pant, D.B. Saru, U.N. Yadav, D.P. Khanal, Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh, J. Intercult. Ethnopharmacol. 6 (2) (2017) 170–176, https://doi.org/10.5455/jice.20170403094055.
[31] R. Masuda, J. Ajimi, T. Murata, Pharmacotherapy for neuropathic pain in Japan, J. Nippon Med. Sch. 84 (6) (2017) 258–267.
[32] G. Oboh, The neuroprotective potentials of sour (*Hibiscus sabdariffa*) and green (*Camellia sinensis*) teas on some pro-oxidants induced oxidative stress in brain, Asian J. Clin. Nutr. 1 (2009) 40–49.
[33] O.J. Efosa, K. Omage, M.A. Azeke, *Hibiscus sabdariffa* calyx protect against oxidative stress and aluminium chloride-induced neurotoxicity in the brain of experimental rats, Toxicol. Rep. (2023), https://doi.org/10.1016/j.toxrep.2023.04.008.
[34] K.J. Meyers, C.B. Watkins, M.P. Pritts, R.H. Liu, Antioxidant and antiproliferative activities of strawberries, J. Agric. Food Chem. 51 (23) (2003) 6887–6892, https://doi.org/10.1021/jf034506n.
[35] I.A. Adedara, S.E. Alake, M.O. Adeyemo, L.O. Olajide, T.O. Ajibade, E.O. Farombi, Taurine enhances spermatogenic function and antioxidant defensemechanisms in testes and epididymis of L-NAME-induced hypertensive rats, Biomed. Pharmacother. 97 (2018) 181–189.
[36] K. Omage, A.M. Azeke, I.I. Ihimire, A.M. Idagan, Phytochemical, proximate and elemental analysis of *Acalypha wilkesiana* leaves, Sci. J. Pure Appl. Sci. 2 (9) (2013) 323–331, https://doi.org/10.14196/sjpas.v2i9.982.
[37] K. Omage, A.M. Azeke, Medicinal potential of *Acalypha wilkesiana* leaves, Adv. Res. 2 (11) (2014) 655–665, https://doi.org/10.9734/AIR/2014/9452.
[38] O.E. Onyekachukwu, A.M. Osagie, O.S. Omage, K. Omage, A.M. Azeke, *Picralima nitida* protects against hepatotoxicity and oxidative stress in alloxan-induced diabetic rats, Comp. Clin. Pathol. 30 (2021) 981–993, https://doi.org/10.1007/s00580-021-03297-y.
[39] O.S. Omage, N.E.J. Orhue, K. Omage, *Dennettia tripetala* combats oxidative stress, protein and lipid dyshomeostasis, inflammation, hepatic injury, and glomerular blockage in rats, Prev. Nutr. Food Sci. 26 (2) (2021) 177–185, https://doi.org/10.3746/pnf.2021.26.2.177.
[40] O.S. Innih, I.G. Eze, K. Omage, Cardiovascular benefits of *Momordica charantia* in cholesterol-fed Wistar rats, Clin. Phytosci. 7 (2021) 65, https://doi.org/10.1186/s40816-021-00303-2.
[41] E.G. Orumwense, A.M. Osagie, O.S. Omage, K. Omage, A.M. Azeke, *Synclisia scabrida* protects against oxidative stress, hepatotoxicity and hyperglycaemia in alloxan-induced diabetic rats, J. Diabetes Metab. Disord. 21 (2022) 669–680, https://doi.org/10.1007/s40200-022-01029-9.
[42] D.W. Cushman, H.S. Cheung, Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung, Biochem. Pharm. 20 (7) (1971) 1637–1648, https://doi.org/10.1016/0006-2952(71)90292-9.
[43] S.H. Audi, N. Friedly, R.K. Dash, A.M. Beyer, A.V. Clough, E.R. Jacobs, Detection of hydrogen peroxide production in the isolated rat lung using Amplex red, Free Radic. Res. 52 (9) (2018) 1052–1062, https://doi.org/10.1080/10715762.2018.1511051.
[44] C. Zhang, T.W. Hein, W. Wang, C.I. Chang, L. Kuo, Constitutive expression of arginase in microvascular endothelial cells counteracts nitric oxide-mediated vasodilatory function, Faseb J. 15 (7) (2001) 1264–1266, https://doi.org/10.1096/fj.00-0681fje.
[45] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide 5 (1) (2001) 62–71.
[46] A. Thawabteh, S. Juma, M. Bader, D. Karaman, L. Scrano, S.A. Bufo, R. Karaman, The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens, Toxins 11 (11) (2019), https://doi.org/10.3390/toxins11110656.
[47] Z. Adamski, L.L. Blythe, L. Milella, S.A. Bufo, Biological activities of alkaloids: from toxicology to pharmacology, Toxins 12 (4) (2020), https://doi.org/10.3390/toxins12040210.
[48] V. Hirunpanich, A. Utaipat, N.P. Morales, N. Bunyapraphatsara, H. Sato, A. Herunsale, C. Suthisisang, Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats, J. Ethnopharmacol. 103 (2) (2006) 252–260, https://doi.org/10.1016/j.jep.2005.08.033.
[49] S. Gosain, R. Ircchiaya, P.C. Sharma, S. Thareja, A. Kalra, A. Deep, T.R. Bhardwaj, Hypolipidemic effect of ethanolic extract from the leaves of Hibiscus sabdariffa L. in hyperlipidemic rats, Acta Pol. Pharm. 67 (2) (2010) 179–184.
[50] M.Y. Yang, C.H. Peng, K.C. Chan, Y.S. Yang, C.N. Huang, C.J. Wang, The hypolipidemic effect of *Hibiscus sabdariffa* polyphenols viainhibiting lipogenesis and promoting hepatic lipid clearance, J. Agric. Food Chem. 58 (2) (2010) 850–859, https://doi.org/10.1021/jf903209w.
[51] M. Yakubu, M.A. Akanji, T. Oladiji, Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem, Res. J. Med. Plant 2 (2008) 66–73.
[52] J.S. Josiah, C.O.S. Nwangwu, K. Omage, A.A. Abdulrahamon, B. Nkwonta, O. F. Aderoju, Possible revival of atrophied islet cells of the pancreas by *Vernonia amygdalina* in alloxan induced diabetic rats, J. Appl. Pharm. Sci. 2 (9) (2012) 127–131, https://doi.org/10.7324/JAPS.2012.2925.
[53] M.F.M. Donfack, A.D. Atsamo, R.J.T. Guemmogne, O.B.N. Kenfack, A.B. Dongmo, T. Dimo, Antihypertensive effects of the *Vitex cienkowskii* (Verbenaceae) stem-bark

extract on L-NAME-induced hypertensive rats, Evid.-Based Complement. Altern. Med. (2021), https://doi.org/10.1155/2021/6668919.
[54] K.K. Gauthaman, M.T. Saleem, P.T. Thanislas, V.V. Prabhu, K.K. Krishnamoorthy, N.S. Devaraj, J.S. Somasundaram, Cardioprotective effect of the Hibiscus rosa sinensis flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat, BMC Complement. Altern. Med. 6 (2006) 32, https://doi.org/10.1186/1472-6882-6-32.
[55] H.A.H. Khattab, N.A. El-Shitany, A.K.S. Al-lily, Effect of Roselle (Hibiscus sabdariffa) against adriamycin induced-cardiotoxicity in male rats, Int. J. Pharm. Res. Allied Sci. 5 (3) (2016) 519–534.
[56] S.K. Banerjee, A.K. Dinda, S.C. Manchanda, S.K. Maulik, Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury, BMC Pharm. 2 (2002) 16, https://doi.org/10.1186/1471-2210-2-16.
[57] A.E. Godwill, Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases, in: A. Toshiki, A. Md (eds.), Phytochemicals, 2018, pp. Ch. 4.
[58] O. Pechanova, S. Vrankova, M. Cebova, Chronic L-NAME-treatment produces hypertension by different mechanisms in peripheral tissues and brain: role of central eNOS, Pathophysiology 27 (1) (2020) 46–54, https://doi.org/10.3390/pathophysiology27010007.
[59] B. Shaukat, M.H. Mehmood, B. Murtaza, F. Javaid, M.T. Khan, M. Farrukh, R. Rana, M. Shahzad, Ajuga bracteosa exerts antihypertensive activity in L-NAME-induced hypertension possibly through modulation of oxidative stress, proinflammatory cytokines, and the nitric oxide/cyclic guanosine monophosphate pathway, ACS Omega 7 (37) (2022) 33307–33319, https://doi.org/10.1021/acsomega.2c03888.
[60] C. Udenigwe, L. Yin-shiou, W.C. Hou, R. Aluko, Kinetics of the inhibition of renin and angiotensin I-converting enzyme by flaxseed protein hydrolysate fractions, J. Funct. Foods 1 (2009) 199–207, https://doi.org/10.1016/j.jff.2009.01.009.
[61] L. Paiva, E. Lima, A.I. Neto, J. Baptista, Angiotensin I-converting enzyme (ACE) inhibitory activity, antioxidant properties, phenolic content and amino acid profiles of *Fucus spiralis* L. protein hydrolysate fractions, Mar. Drugs 15 (10) (2017), https://doi.org/10.3390/md15100311.
[62] R.F. Abdel-Rahman, A.F. Hessin, M. Abdelbaset, H.A. Ogaly, R.M. Abd-Elsalam, S. M. Hassan, Antihypertensive effects of Roselle-olive combination in L-NAME-induced hypertensive rats, Oxid. Med. Cell. Longev. 2017 (2017) 9460653, https://doi.org/10.1155/2017/9460653.
[63] G. Gallo, M. Volpe, C. Savoia, Endothelial dysfunction in hypertension: current concepts and clinical implications, Front. Med. 8 (2022), 798958, https://doi.org/10.3389/fmed.2021.798958.
[64] M.R. Weir, V.J. Dzau, The renin-angiotensin-aldosterone system: a specific target for hypertension management, Am. J. Hypertens. 12 (3) (1999) 205s–213s, https://doi.org/10.1016/s0895-7061(99)00103-x.
[65] A. Ahmad, S.K. Dempsey, Z. Daneva, M. Azam, N. Li, P.I. Li, J.K. Ritter, Role of nitric oxide in the cardiovascular and renal systems, Int. J. Mol. Sci. 19 (9) (2018), https://doi.org/10.3390/ijms19092605.
[66] P.I. Mount, D.A. Power, Nitric oxide in the kidney: functions and regulation of synthesis, Acta Physiol. 187 (4) (2006) 433–446, https://doi.org/10.1111/j.1748-1716.2006.01582.x.