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Formulated Polyherbal Tea Upregulates Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) Gene Expression and Optimizes antioxidant Biomarkers in Diabetic-Hypertensive Rats

Idowu Sunday Oyeleye^{1,2*}, Olajide Raymond Ojo¹, Opeyemi Babatunde Ogunsuyi^{1,2}, Adedayo Oluwaseun Ademiluyi¹ and Ganiyu Oboh^{1*}

Abstract

Background The rising incidence of hypertension and diabetes is among the contributors to global death, with a significant financial burden. This study aims to explore the popularity of plant-based substitutes, focusing on a tea-based polyherbal blend of Roselle calyx, *Moringa oleifera*, and *Annona muricata* leaves, and its effect on the NRF2 gene and enzymes linked to diabetes and hypertension.

Method The samples (1 g, containing a 2:2:6 ratio of the plant) were weighed into tea bags with or without aspartame as a sweetener, and then steeped in 100 ml of boiling water for 5, 10, and 15 minutes. Adult albino rats were fed a high-fat diet and received Streptozotocin STZ (35 mg/kg b.w.). Diabetes (glucose > 250 mg/dl) was confirmed after 72 hours, followed by L-NAME (40 mg/kg/day) administration and sample treatment. Nuclear factor erythroid 2-related factor 2 gene, antioxidant enzymes (superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST), glutathione peroxidase (GPX), and diabetes-hypertension-linked enzymes: purinergic (ATP, AMP, and ADA), arginase, phosphodiesterase (PDE-5), angiotensin-converting enzyme (ACE) were determined.

Results The results revealed a decrease in PDE-5, arginase, ACE, ATP, AMP, and ADA activities by the treatment. The study also demonstrates that polyherbal tea has a beneficial effect by upregulating the NRF2 gene, leading to the amelioration of oxidative stress through the SOD, catalase, GST, and GPX pathways.

Conclusion

These results highlight the potential of polyherbal tea with the longest brewing times as a viable management strategy for diabetes and hypertension.

*Correspondence:

Idowu Sunday Oyeleye

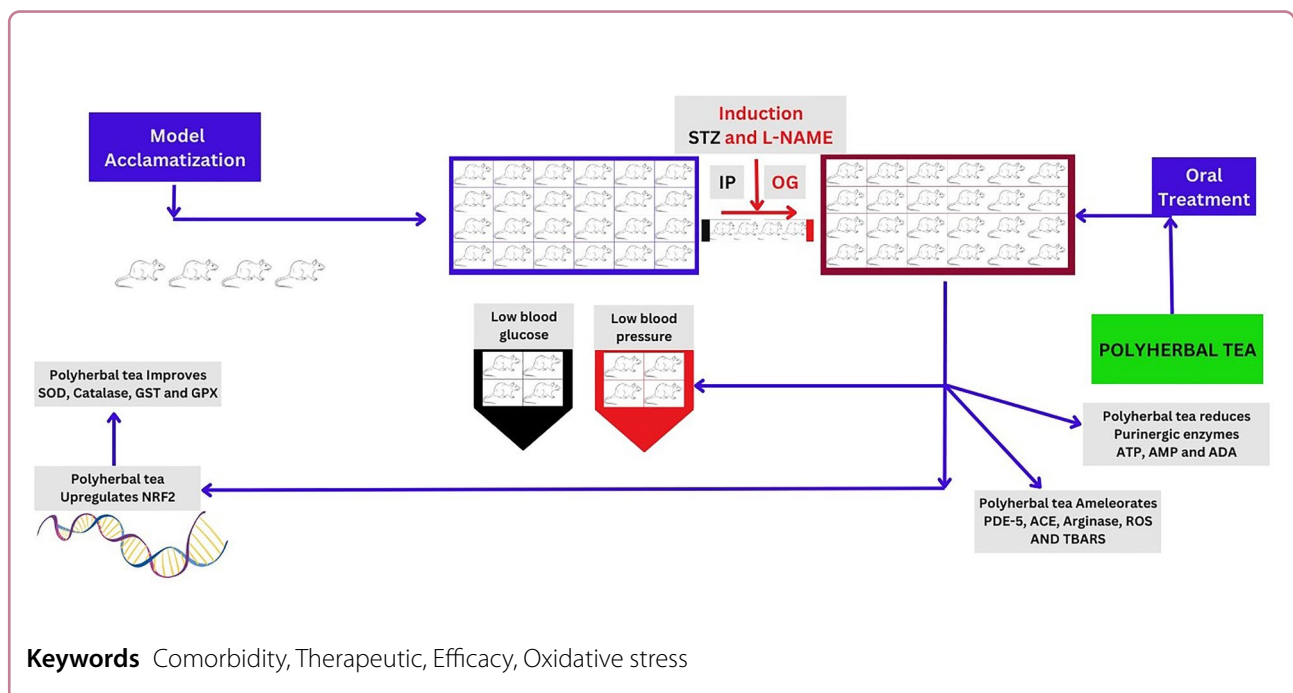
sioyeleye@futa.edu.ng

Ganiyu Oboh

goboh2001@yahoo.com

Full list of author information is available at the end of the article

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1 Introduction

The prevalence of diabetes mellitus (DM) and hypertension (HYP) and their co-morbidity, particularly in Western society, is on the increase with age [1–3]. The coexistence of DM and HYP significantly raises morbidity and mortality rates, contributing to a substantial economic burden on society [4]. The estimated annual costs for DM and HYP-related issues are \$174 billion and \$76.6 billion, respectively [5, 6]. The use of acarbose as an oral inhibitor has potential benefits in managing pre-diabetes, type 2 diabetes mellitus, and obesity by controlling blood sugar levels, thus controlling diabetes-related complications [7]. The shift towards plant-based alternatives is largely based on their safety and efficacy [8, 9].

Tea originated in China over 60 million years ago, with records of tea drinking dating back approximately 3000 years. Today, it's popular, medicinal, and culturally rich, providing access to the entire world to explore in various ways for the management of diseases such as diabetes and hypertension [10–13]. The emphasis on the potential of herbal plants, especially when combined, for synergistic effects has become an exploratory ground [14, 15]. It introduces the concept of using plant phytochemicals to develop treatments for DM and its consequences [16, 17]. Tea, known for its various bioactivities, is being explored for its potential to mitigate the risk of diabetes and its complications, owing to its bioactive components, such as polyphenols [18, 19]. Studies on herbal tea emphasise the benefits of polyherbal formulations, citing specific plants such as Roselle (*Hibiscus sabdariffa*),

Moringa oleifera, and *Annona muricata* (soursop) leaves [11, 18–20]. These plants are noted for their anti-inflammatory, antioxidant, and anti-hyperglycemic properties [21–23].

Across the entire field of plant research, several plants have been studied for their potential in managing diabetes, oxidative stress, and hypertension. Among the top researched plants are *Hibiscus sabdariffa* (Roselle), *Moringa oleifera*, and *Annona muricata* (soursop). Soursop leaf is widely recognized as an alternative treatment for hypertension and is noted for its effectiveness in managing both diabetes and hypertension. Research conducted by Adefegha et al. [24] and Tran et al. [25] has highlighted the antihypertensive properties of soursop. Similarly, Luqman et al. [26] and Adejoh et al. [27] studied *Moringa* leaf, which is noted for its antioxidant properties that help moderate diabetes, ultimately recommending *Moringa* for diabetes management. Hibiscus flowers are notable for their abundance of polyphenolic compounds, with key identified components including anthocyanins and flavonoids such as quercetin, kaempferol, and their derivatives. Oboh et al. [28] demonstrated that Roselle inhibits α -amylase and α -glucosidase activities while exhibiting significant antioxidant properties. Diabetes and hypertension frequently co-exist, sharing a multifactorial pathophysiology. Although lifestyle changes and conventional medications are the primary treatment approaches, tea's potential as a dietary intervention remains underexplored. While individual research has been conducted on the effects of soursop, *Moringa*,

and Roselle, their combined effects have yet to be studied. This study aims to evaluate the impact of a formulated Polyherbal Tea blend of Roselle calyx and the leaves of Moringa and Soursop on nuclear factor erythroid 2-related factor 2 (NRF2) gene expression, antioxidant biomarkers, and the purinergic system in diabetic hypertensive rats, as well as their hemodynamic status. The efficacy of the formulated teas over different infusion times was also considered.

2 Materials and Methods

2.1 Chemicals

L-NAME (NG-nitro-L-arginine methyl ester hydrochloride) and Streptozotocin (STZ) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Distilled water (DW) was used to prepare the reagents. All other chemicals used in this experiment were of analytical grade.

2.2 Sample Preparation

The calyx of the Roselle was obtained from Akure Market Square, while the leaves of the Soursop and Moringa were freshly collected from the University Botanical Garden. The Centre for Research and Development at the Federal University of Technology, Akure, approved the use. Each sample was freeze-dried to a constant weight and ground into powder using a Waring blender (Waring® CB15). Afterwards, the powder was sieved through an Aldrich fine test SS frame sieve (Z400122) with a 125 µm pore size. The samples with a total tea content of 1 g in a 2:2:6 ratio (Moringa: Soursop: Roselle Calyx) were weighed and bagged. To compensate for the unpleasant taste, aspartame was added to one portion and absent in the other. In a covered teacup filled with 100 ml of boiling water, a tea bag (sweetened/unsweetened) was steeped

for 5, 10, and 15 min, respectively. The tea was served cooled to the rats during treatment.

2.3 Experimental Design and Animal Handling

The Ethical Committee of the Federal University of Technology, Akure, approved the use and handling of the animal by the National Institutes of Health (2011) guidelines, with reference number FUTA/SOS/1411. Sixty-three adult male albino Wistar rats, weighing between 235 and 250 g, were obtained from the Department of Animal Production and Health at the Federal University of Technology, Akure, Nigeria. Before inducing diabetes, they were given a two-week acclimatization period and fed a high-fat diet for 14 days (Table 1). After an overnight fast, they received a single intraperitoneal (i.p.) injection of 35 mg/kg body weight of freshly prepared STZ dissolved in citrate buffer (0.1 M, pH 4.5) [9]. The control rats received only citrate buffer. After 72 h, a glucose test kit confirmed the animals' blood glucose levels. Rats with blood glucose levels of 250 mg/dl or above were considered diabetic. The diabetic rats received L-NAME at a dose of 40 mg/kg/day for 10 days [29, 30]. Systolic and diastolic blood pressure were measured using a tail-cuff device [29]. The rats were grouped into nine groups ($n=7$) based on their treatment regimens as described below:

- Group 1: Normal control received citrate buffer (pH 4.5).
- Group 2: DM-HYP induced rats (DM + HYP).
- Group 3: DM-HYP + ACA + Lsprl)
- Group 4: DM-HYP + sweetened PHT infused for 5 min treatment.
- Group 5: DM-HYP + unsweetened PHT (5 min) treatment.
- Group 6: DM+HYP+sweetened PHT (10 min) treatment.
- Group 7: DM+HYP+unsweetened PHT (10 min) treatment.
- Group 8: DM+HYP sweetened PHT (15 min) treatment.
- Group 9: DM+HYP unsweetened PHT (15 min) treatment.

2.4 Blood Sample Tissue Collection, and Preparation

On day 15, rats were anaesthetised with pentobarbital, euthanised, and dissected, and blood was rapidly collected into plain tubes devoid of protease inhibitors. The tubes were spun on an MSC bench centrifuge (Beckman Coulter, Fullerton, CA, USA) for 15 min at 5000 rpm. The pancreas and intestines were rapidly removed, cleaned in cold saline, weighed on ice, and then homogenised in sodium phosphate buffer (0.1 M, pH 7.4). A portion was

Table 1 Primer sequence

S/N	Gene name	Accession No.	Sequence
1	β-actin	Nm_031144.3	Forward: GTGTGGATTGGTGGCTCTATC Reverse: CAGTCCGCCTAGAAGCATTT
2	GaPDH	Nm_017008.4	Forward: CATCTCCCTCACAATTCATCC Reverse: GAGGGTGCGACGGAACTTTAT
3	Nrf2	Nm_031789.2	Forward: CTTCCTCTGCTGCCATTAGT Reverse: CAGTGTGCTTCTGTTGAAAG

kept in Trizol for RNA expression. The clear supernatant was stored at -20°C for biochemical analysis.

2.5 Determination of Glycated Haemoglobin (HbA1c) Levels

The study of Aloud et al. [31] detailed the procedure for determining GHb levels [32, 47].

2.6 Biochemical Assays

Using Bradford's method, Coomassie blue and bovine serum albumin were used to determine the protein concentration of pancreas tissue [33]. Using the substrate hippuryl-L-histidyl-L-leucine (HHL) and the spectrophotometric method of Bakhle [34] at 228 nm, the activity of the angiotensin-I-converting enzyme (ACE) was assessed.

PDE-5 activity was determined as described by Kelly and Buttler [35], with modifications by Oyeleye et al. [36]. Arginase activity was determined by measuring the rate of urea production using α -isonitrosopropiophenone (9% in absolute ethanol), as described by Geyer and Dabich [37]. The NTPDase enzymatic assay of the tissue homogenate was performed using the method described by Schetinger et al. [38]. The 5'-nucleotidase activity was measured as described by Heymann et al. [39]. Adenosine deaminase (ADA) activity was investigated according to the previously established methods [40]. SOD activity in the supernatant was assessed using the methods of Misra and Fridovich [41], as described by Oyeleye et al. [36]. The activity of SOD was estimated by measuring adrenochrome formation at 480 nm and 25°C in a reaction

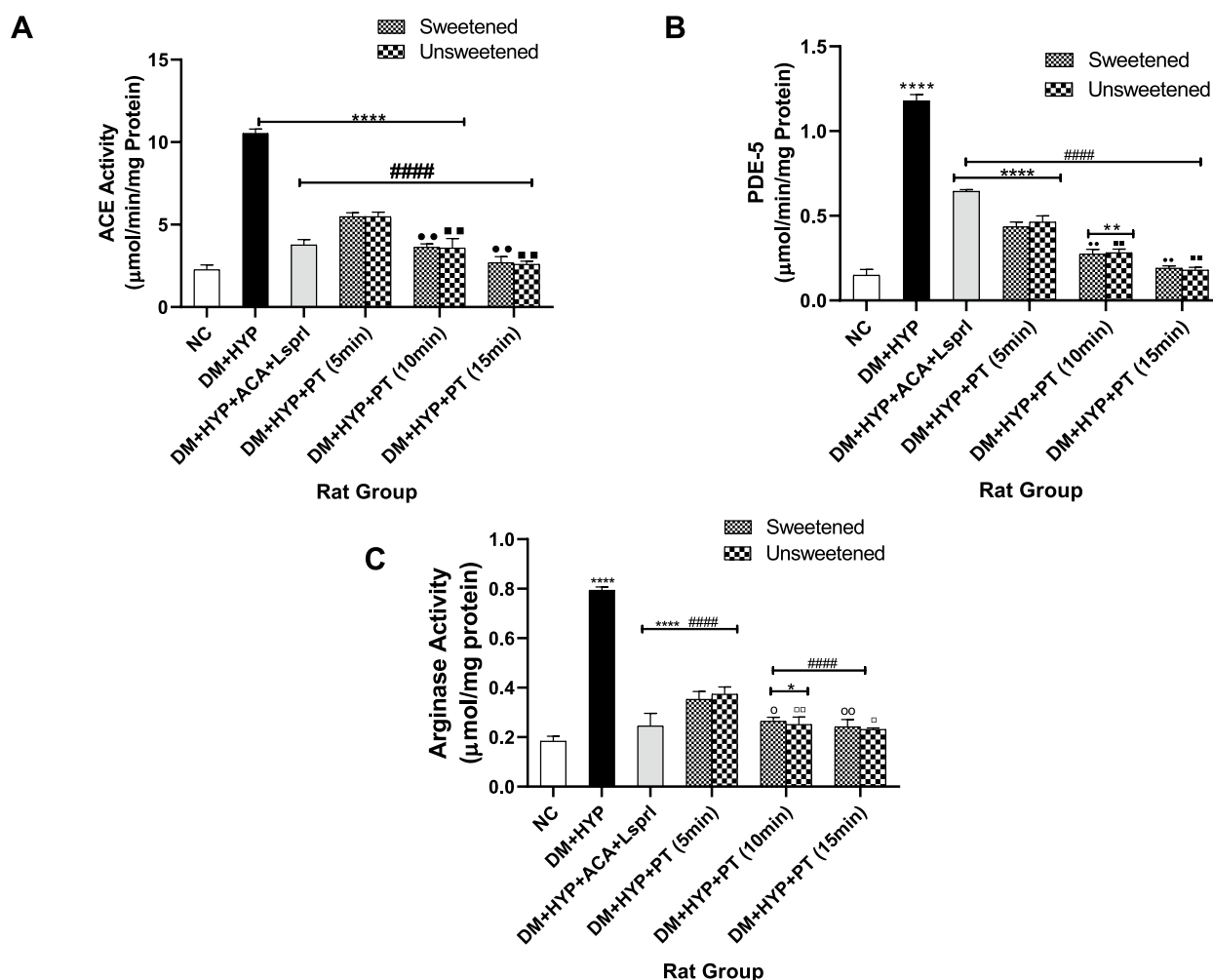


Fig. 1 Effect of Polyherbal tea on **A** Angiotensin converting enzyme (ACE) **B** Phosphodiesterase –5 enzyme (PDE-5) and **C** Arginase enzyme activity in DM + HYP induced Male rat. Bars are represented as mean $\pm$ SEM ($n=7$). Values are significantly different ** $p < 0.01$ **** $p < 0.0001$ versus NC; #### $p < 0.0001$ versus DM + HYP; $op < 0.05$, $oop < 0.01$, ●● $p < 0.0001$ versus DM + HYP + PT (5 min) sweetened; □□ $p < 0.05$, □□ $p < 0.01$, ■■ $p < 0.0001$ versus DM + HYP + PT (5 min) unsweetened. NC Normal control, DM-HYP Diabetes-hypertensive, ACA + Lspri Acabose + Lisinopril; PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

mixture containing glycine–NaOH (50 mM, pH=10.0) and adrenaline (1 mM) [41]. The activities of CAT, GST, and GPx were also determined [42, 43]. The TBARS assay was performed using the method described by Ohkawa et al. [44]. The ROS level in the tissue homogenate was determined using the methodology of Hayashi et al. [45], as reported by Ademosun et al. [46].

3 Molecular Procedure

3.1 Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) and RNA Extraction

Trizol[®] was used to extract total RNA from the pancreas of the rats in each group. DNase I treatment, 1.5% agarose gel visualization, and RNA quantification using the Nanodrop 2000 (Invitrogen) were performed on the samples. cDNA was synthesised using the iScript[™] cDNA Synthesis Kit to yield 1 µg of cDNA. Insulin was selected as the reference gene for all expression analyses. To

complete the reactions, which were carried out in a final volume of 20 µL with 2.5 ng/µL of cDNA, 1×PCR buffer, 0.2 µM of the primer (Table 1), 0.2 mM dNTP, 2 mM MgCl₂, and 0.1×SYBR[®] Green, 40 heat cycles of 15 s at 94 °C, 10 s at 60 °C, and 30 s at 72 °C were applied [29]. SYBR fluorescence was measured using the StepOne[™] design and analysis software. All experiments were performed in triplicate (n=5). The 2^{−ΔΔCT} method [48] was used to calculate gene expression levels.

3.2 Statistical Analysis

All the data were expressed as the mean and standard error of the mean. Using GraphPad Prism 8.0.2 software, a two-way analysis of variance (ANOVA) was conducted to evaluate the differences between the groups.

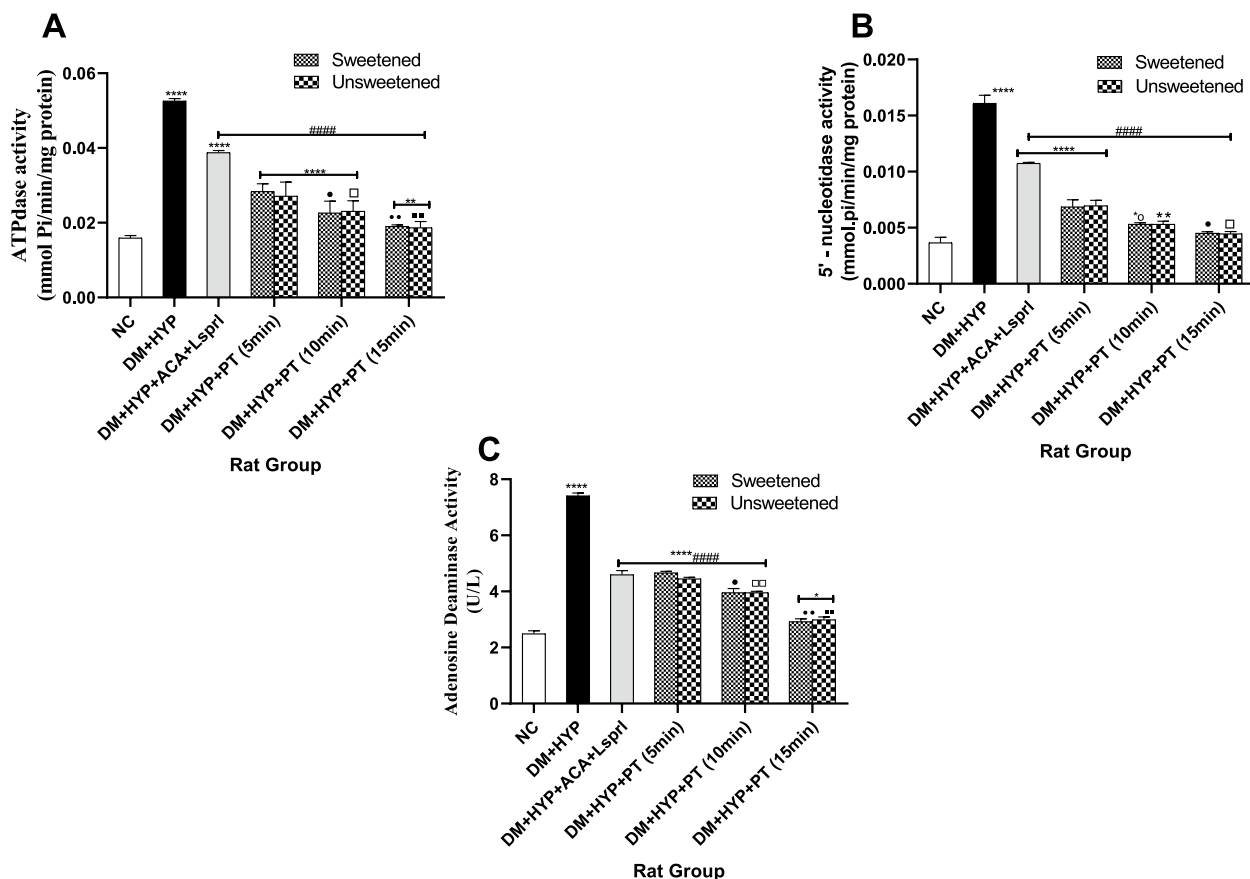


Fig. 2 Effect of Polyherbal tea on **A** ATPase **B** 5' Nucleotidase and **C** Adenosine Deaminase activity in DM+HYP induced male rat. Bars are represented as mean ± SEM (n=7). Values are significantly different * $p < 0.05$, ** $p < 0.01$ **** $p < 0.0001$ versus NC; #### $p < 0.0001$ versus DM+HYP; op $p < 0.05$, oop $p < 0.01$, ● $p < 0.001$, ●● $p < 0.0001$ versus DM+HYP+PT (5 min) sweetened; □□ $p < 0.05$, □□ $p < 0.01$, ■■ $p < 0.0001$ versus DM+HYP+PT (5 min) unsweetened. NC Normal control, DM+HYP Diabetes-hypertensive, ACA+Lsprl Acabose+Lisinopril, PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

4 Results

The DM+HYP group, which exhibited a significant increase ($*** p < 0.0001$) in ACE activity, compared to a control group with unaltered ACE enzyme activity (Fig. 1A). Although there was no discernible difference between sweetened and unsweetened varieties.

Interestingly, both sweetened and unsweetened PHT showed a substantial decrease ($\bullet\bullet p < 0.0001$) at 10 and 15 min compared to 5 min. The ACE result also complemented the hemodynamic findings in Supplementary Fig. 8 (Systolic and Diastolic), where the PHT reduces blood Pressure parameters.

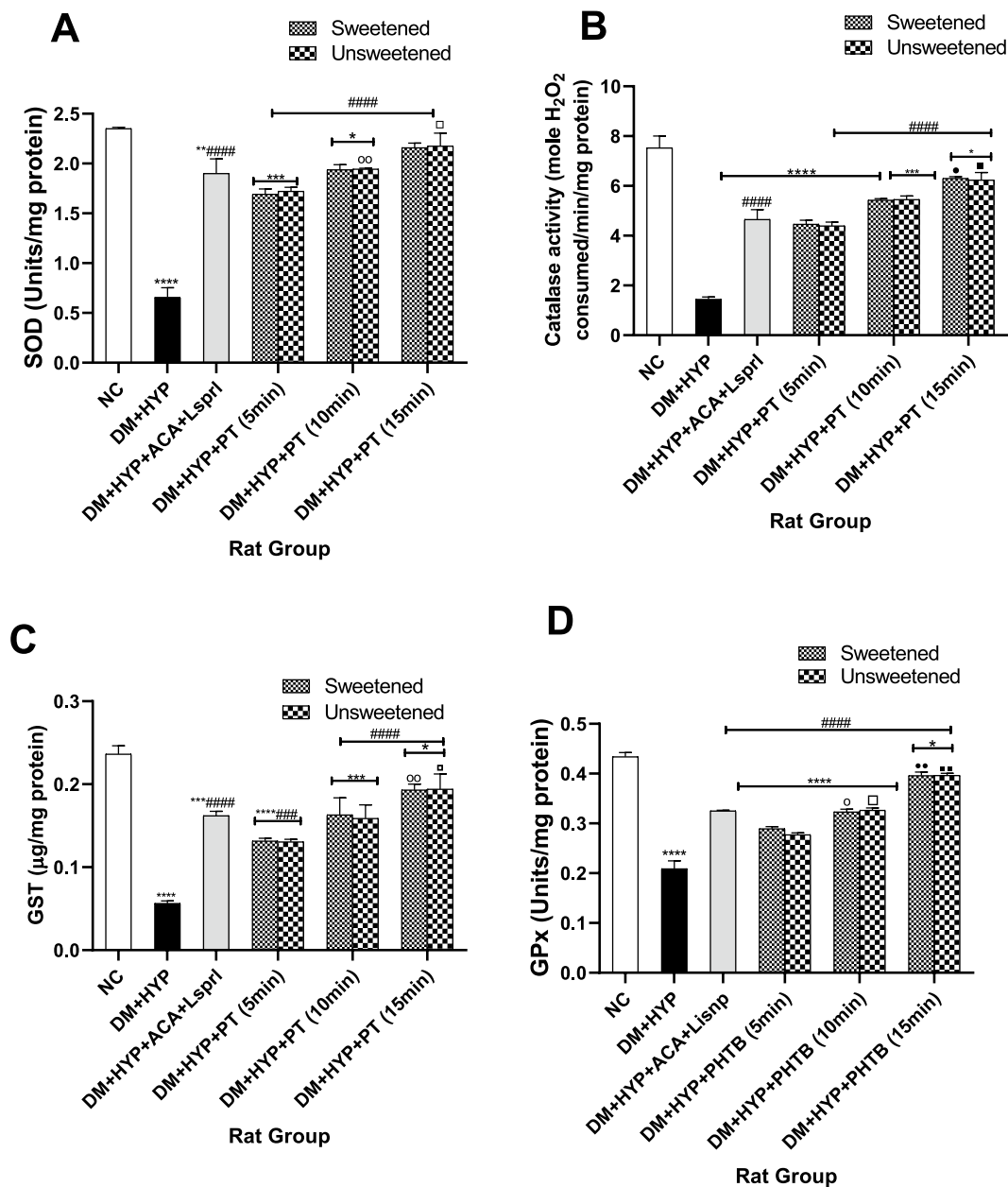


Fig. 3 Effect of Polyherbal tea on **A** Superoxide Dismutase (SOD) **B** Catalase **C** Glutathione-S Transferase (GST) **D** Glutathione Peroxidase (GPx) of DM+HYP induced male rat. Bars are represented as mean $\pm$ SEM ($n = 7$). Values are significantly different $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ versus NC; $###p < 0.001$, $####p < 0.0001$ versus DM+HYP; $op < 0.05$, $oop < 0.01$, $\bullet p < 0.001$, $\bullet\bullet p < 0.0001$, versus DM+HYP+PT (5 min) sweetened; $\square p < 0.01$, $\blacksquare p < 0.001$, $\blacksquare\blacksquare p < 0.0001$ versus DM+HYP+PT (5 min) unsweetened. NC Normal control, DM+HYP Diabetes-hypertensive, ACA + Lspri Acabose + Lisinopril; PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

Arginase enzyme activity was considerably higher ($****p < 0.0001$) in the DM+HYP group than in the control group, as shown in Fig. 1 B. Although there is no effect of the sweetener between the treatment groups ($#### p < 0.0001$). Compared to their 5-min counterparts, arginase activity decreased in both the sweetened and unsweetened groups after administration of PTH infused for 10 and 15 min. PDE-5 activity was significantly higher in DM+HYP rats ($****p < 0.0001$) than in the control group (Fig. 1C). PDE-5 activity was considerably reduced by PHT treatment ($####p < 0.0001$), with further decreases observed in the treatment with 10 and 15 min ($●●p < 0.0001$ for sweetened; $■■p < 0.0001$ for unsweetened) infused PTH, compared to that of 5 min. Again, there was no discernible difference between the sweetened and unsweetened groups. In general, PHT with/without sweetener reduced PDE-5, ACE, and arginase activities, with the effects higher in rats treated with 15-min infused PTH.

Figure 2A shows a significant increase ($****p < 0.0001$) in ATPase activity in the DM+HYP group compared to the normal control (NC). Treatment with PHT, both sweetened and unsweetened, had a reduction in ATPase activity. Notably, the 15-min treatment significantly reduced activity ($●●p < 0.0001$; $■■p < 0.0001$) compared to the 5-min infused tea treatment, while the 10-min treatments also showed reductions ($●p < 0.001$; $□p < 0.01$). In Fig. 2B, 5'Nucleotidase activity was lower in all PHT-treated groups and slightly reduced in ACA+Lisnp compared to DM+HYP ($####p < 0.0001$). Significant differences were observed between 10 min ($op < 0.05$)

and 15 min ($●p < 0.001$; $□p < 0.01$) PHT treatments compared to the 5 min treatment. Figure 2C illustrates ADA activity, which increased significantly in DM+HYP rats ($****p < 0.0001$) but was reduced in all treatment groups. ACA+Lisnp and PHT (5, 10, and 15 min) significantly reduced ADA activity ($####p < 0.0001$), with the 15 min infused PHT treated group having stronger reductions ($●●p < 0.0001$; $■■p < 0.0001$) and the 10 min PHT treatment showing moderate reduction ($●p < 0.001$; $□p < 0.05$) compared to 5 min treatments.

Figure 3A shows significantly higher SOD activity in the normal control (NC) group, while the DM+HYP group exhibited reduced activity ($****p < 0.0001$). After 14 days of PHT treatment, both sweetened and unsweetened forms showed improved SOD activity, with no significant difference between them. However, activity increased with treatment duration ($####p < 0.0001$). Comparing 5 min to 10 and 15 min of infused tea, the unsweetened PHT group showed a significant difference ($p < 0.01$), while the sweetened groups showed no such trend. Figure 3B reveals that catalase activity was significantly lower in the DM+HYP group compared to NC ($****p < 0.0001$), while PHT treatments improved catalase activity ($####p < 0.0001$). The 15-min infused tea (with/without sweetened) treated rats showed significantly higher catalase activity ($●p < 0.001$; $■p < 0.001$) compared to the control. In Fig. 3C, GST activity was reduced in the DM+HYP group ($****p < 0.0001$) but increased with PHT treatment ($####p < 0.0001$; $####p < 0.0001$), and the group treated with 15-min infused PTH had significantly higher activity ($oop < 0.01$; $□p < 0.01$). Figure 3D indicates

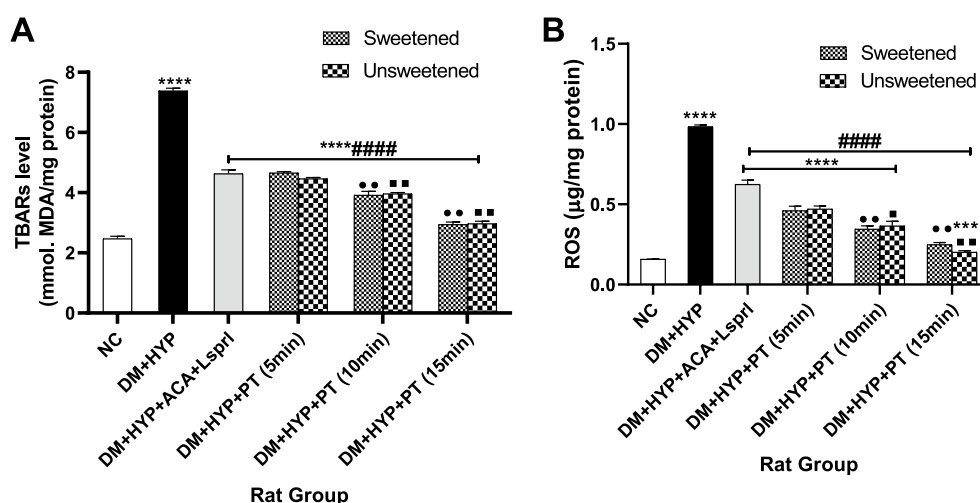


Fig. 4 Effect of Polyherbal tea blend on **A** TBARS Level **B** ROS level in pancreas of diabetic—hypertensive male rat. Bars are represented as mean $\pm$ SEM ($n = 7$). Values are significantly different $***p < 0.001$, $****p < 0.0001$ versus NC; $####p < 0.0001$ versus DM+HYP; $●●p < 0.0001$, versus DM+HYP+PT (5 min) sweetened; $■■p < 0.001$, $■■p < 0.0001$ versus DM+HYP+PT (5 min) unsweetened. NC Normal control, DM+HYP Diabetes-hypertensive, ACA+Lsnp Acabose+Lisinopril, PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, US PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

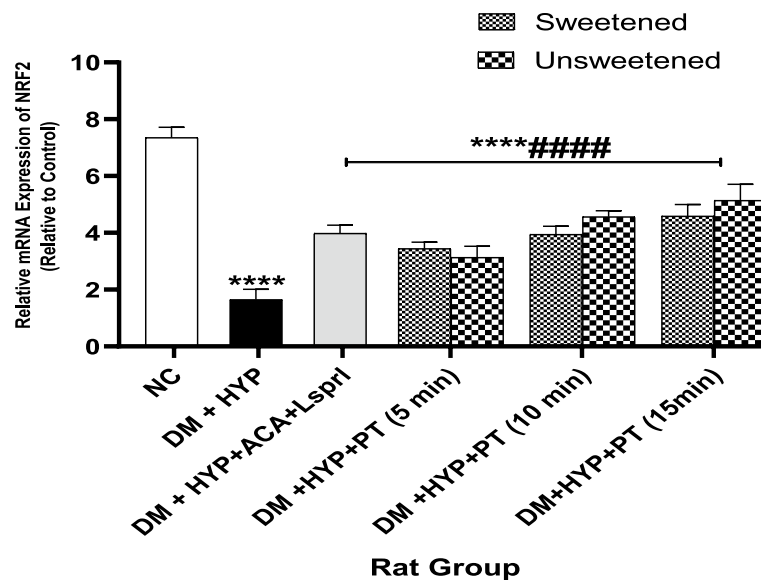


Fig. 5 Effect of Polyherbal Tea Blend on NRF2 Gene Expression in the Pancreas of diabetic—hypertensive male rat. Bars are represented as mean $\pm$ SEM ($n = 7$). Values are significantly different **** $p < 0.0001$ versus NC; #### $p < 0.0001$ versus DM + HYP. NC Normal control, DM-HYP Diabetes-hypertensive, ACA + Lspri Acabose + Lisinopril, PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, US PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

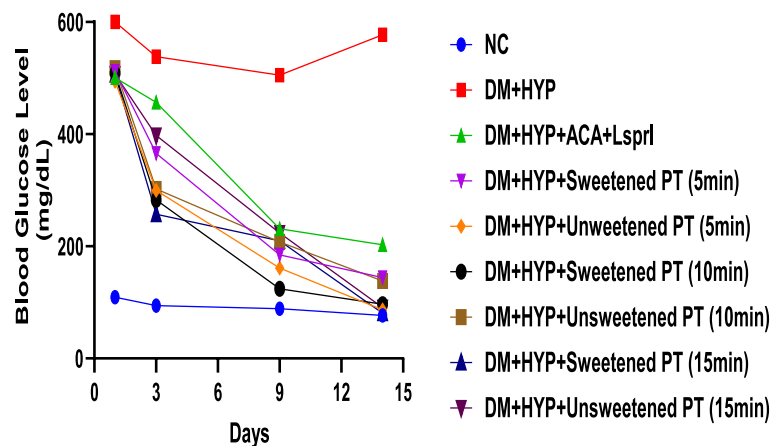


Fig. 6 Effect of Polyherbal tea on Fasting blood glucose level (FGBL) of DM+HYP-induced male rats. Bars are represented as mean $\pm$ SEM ($n = 7$). NC Normal control, DM-HYP Diabetes-hypertensive, ACA + Lspri Acabose + Lisinopril, PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

GPX activity, which was (**** $p < 0.0001$) reduced in the DM+HYP rats' group but improved with ACA+Lispn and PHT treatments. Overall, PHT treatment improved antioxidant enzyme activities regardless of whether sweetened or not.

The DM+HYP group exhibited significantly higher levels of TBARS and ROS (**** $p < 0.0001$) when compared to the NC group (Fig. 4A). Notably, TBARS levels were markedly reduced (#### $p < 0.0001$) in the ACA + lisnp and PHT-treated groups. As the duration of

infusion increased, a significant difference was observed between the 10 and 15-min treatments (●● $p < 0.0001$) in comparison to the 5-min sweetened PHT, with a similar reduction evident in the unsweetened group (■ $p < 0.0001$) when contrasting the 10 and 15-min treatments to that of the 5-min. Furthermore, as illustrated in Fig. 4B, the 15-min sweetened/unsweetened PHT-treated group demonstrated a greater reduction in ROS levels (●● $p < 0.0001$ and ■ $p < 0.0001$) compared to the 5-min sweetened/unsweetened group. While

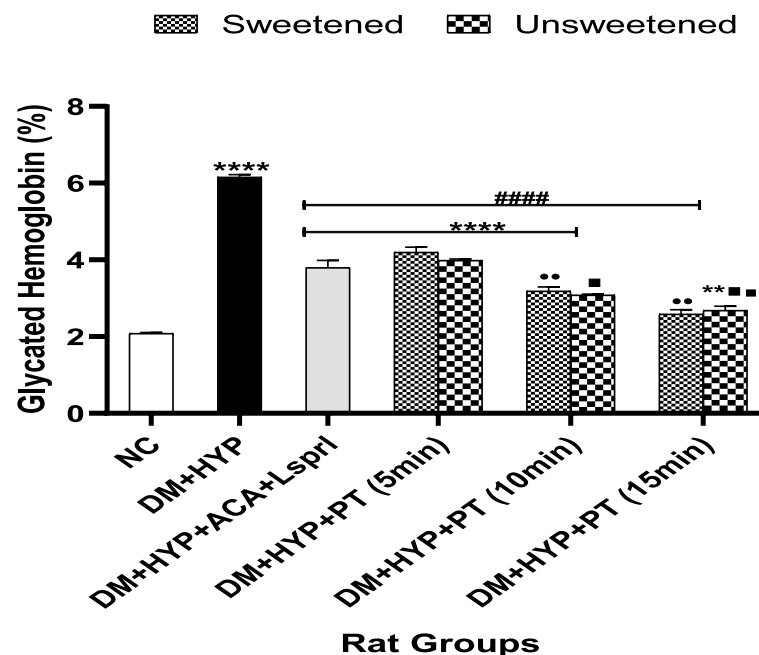


Fig. 7 Effect of Polyherbal tea on Glycated hemoglobin level of DM + HYP-induced male rats. Bars are represented as mean $\pm$ SEM ($n = 7$). Values are significantly different ** $p < 0.01$, **** $p < 0.0001$ versus NC; #### $p < 0.0001$ versus DM + HYP; ●● $p < 0.0001$, versus DM + HYP + PT (5 min) sweetened; ■ $p < 0.001$, ■■ $p < 0.0001$ versus DM + HYP + PT (5 min) unsweetened. NC Normal control, DM-HYP Diabetes-hypertensive, ACA + Lsprl Acabose + Lisinopril, PT Sweetened Polyherbal Tea infused for 5, 10 and 15 min, PT Un-sweetened Polyherbal Tea infused for 5, 10 and 15 min

the 10-min sweetened/unsweetened treatment also resulted in a reduction of ROS levels (●● $p < 0.0001$ and ■ $p < 0.001$), the effect observed was not as pronounced as that observed with 5-min-infused treated rats.

Figure 5 shows that the DM + HYP group had significantly lower NRF2 gene expression in pancreatic tissue compared to the NC group (**** $p < 0.0001$). ACA and Lsprl treatments resulted in a modest increase in NRF2 expression, while the 5-min PHT treatment led to a moderate rise, and the 10-min PHT treatment showed a slightly higher increase. The 15-min PHT group had the highest NRF2 expression among all treatments compared to DM + HYP. The NC group demonstrated markedly higher NRF2 levels than both treated and untreated groups.

Figure 6 illustrates the results of fasting blood glucose levels (FBGL). On the first day, an increase in FBG was noted due to STZ induction, while the control group maintained a consistently low FBG level. As the treatment progressed, the DM + HYP group did not exhibit a reduction in FBGL, unlike the treated group. By the third day, a significant decrease in FBGL was observed in the treatment groups. Additionally, between the ninth and fourteenth days, a stable FBGL level was maintained, indicating that the rats were more stable compared to the DM + HYP group. Figure 7 displays the

impact of DM + HYP and treatment groups on glycated haemoglobin levels. An increase in glycated haemoglobin was recorded in the DM + HYP group compared to the NC group, with a highly significant difference (**** $p < 0.0001$). Minor changes were noted in the ACA + Lsprl and 5-min sweetened/unsweetened PHT treatment groups in comparison to the DM + HYP group. The 10-min and 15-min PHT sweetened/unsweetened treatments showed no significant difference compared to the NC group but demonstrated a highly significant reduction when compared to the DM + HYP group.

Supplementary Fig. 9 presents the serum levels of AST, ALT, and creatinine across all groups. The DM + HYP group exhibited a higher elevation in these markers compared to the NC group. However, there were slight but statistically significant differences in these markers among the treatment groups compared to the control, suggesting potential cellular damage. This indicates that PHT administration at the dosage used did not have an adverse impact on hepatic or renal function.

5 Discussion

The in vitro antioxidant properties and phytochemical composition of moringa leaves highlight their rich content of bioactive compounds and impressive antioxidant strength, which contribute to their antidiabetic effects

[49, 50]. Additionally, previous studies have demonstrated that the leaves of *Annona muricata* are rich in phenolic compounds and exhibit antihypertensive properties by inhibiting angiotensin-converting enzymes in vitro [51–54]. Similarly, *Hibiscus sabdariffa* has been reported to inhibit alpha-amylase and alpha-glucosidase, which are key enzymes in carbohydrate metabolism and blood glucose regulation [55–57]. Health officials have shown concern about the spontaneous increase in diabetes mellitus and its comorbidities, such as hypertension [58]. However, as noted in the study by Oyeleye et al. [9], finding effective management options for metabolic diseases is essential, and polyherbal tea interventions offer an alternative approach to managing and treating diabetes and its complications [9, 59–61].

This study's findings demonstrated that polyherbal tea (PHT) effectively reduced fasting blood glucose levels (FBGL) in diabetic-hypertensive (DM-HYP) rats. Figure 7 confirms this by showing a decrease in glycated haemoglobin (HbA), which forms due to glucose-induced protein alterations [62]. In diabetes, elevated serum glucose enhances haemoglobin-glucose interactions, increasing HbA1c levels. Untreated DM-HYP rats exhibited a rise in HbA, directly proportional to FBGL [32, 47]. However, PHT-treated rats showed a significant reduction in both parameters. Notably, PHT with/without sweetener had a more substantial ameliorative effect. The PHT administration also lowered HbA1c. The phenolic composition of PHT contributed to restoring these metabolic parameters to near-normal levels.

ACE is a component of the renin–angiotensin–aldosterone system (RAAS) and plays a vital role in maintaining blood pressure, fluid balance, and electrolyte homeostasis. Insulin secretion and glucose metabolism correlate with pancreatic ACE activity [63]. Figure 1A shows its impact on diabetes management. Because polyherbal tea contains phenolic-rich ingredients such as Roselle calyx, soursop leaf, and Moringa leaf, all of which have bioactive components with anti-diabetic properties. According to this study, PHT inhibited ACE activity, which was investigated to lower blood pressure [29].

PHT's antioxidant and anti-inflammatory properties contribute to the protection of pancreatic beta cells from oxidative stress and damage due to chronic inflammation [62]. Research indicates that ACE inhibitors can reduce inflammation and oxidative stress in various tissues, including the pancreas. By mitigating oxidative stress and inflammation, PHT may help preserve beta cell function and promote insulin production [64]. Additionally, the consumption of PHT may enhance the sensitivity of peripheral tissues, such as skeletal muscle and adipose tissue, to insulin. The effect of ACE inhibition has been associated with reduced insulin resistance and improved

insulin sensitivity, enabling these tissues to utilize glucose more effectively [65].

Figure 1B shows that PDE-5 plays a vital role in regulating blood flow and vascular function. PDE-5 is an enzyme primarily responsible for breaking down cyclic guanosine monophosphate (cGMP) [66, 67]. In diabetes, PDE-5 activity may be increased due to elevated glucose levels, leading to reduced cGMP levels and impaired vascular function [68]. PDE-5 activity may also increase in hypertension, particularly in the pulmonary vasculature [69]. This can contribute to pulmonary hypertension, an elevated blood pressure in the pulmonary arteries [68, 69].

Studies have shown that PHT can potentially reduce arginase activity in individuals with diabetes and hypertension. For instance, a survey by Langarizadeh et al. [70] found that phytochemicals from polyherbal reduced arginase activity and increased NO production in rats. Arginase is an enzyme that plays a crucial role in the urea cycle and the regulation of nitric oxide (NO) production [71]. High levels of arginase activity have been linked to endothelial dysfunction, a key factor in the development of hypertension and diabetes complications [72]. The enzyme arginase is a component of the urea cycle, which breaks down arginine into ornithine and urea [70]. Increased arginase activity has been reported in diabetes, which reduces the amount of arginine available to generate nitric oxide (NO), thereby inducing endothelial and insulin dysfunctions [59, 60, 62]. Interestingly, plants rich in phytochemicals, such as polyphenols, have been demonstrated to inhibit arginase activity, as observed in this study. This implies that PHT could improve NO availability by inhibiting arginase activity and regaining pancreatic vascular function and insulin secretion.

Intriguing findings were observed in the mechanism of arginase and PDE-5 enzymes in the pancreas of diabetic rats and their reaction to PHT administration. One of the changes the pancreas undergoes in diabetes that affects its function is an alteration in enzyme activity [72, 73]. Two enzymes essential in regulating insulin secretion and vascular function are arginase and PDE-5 [74, 75]. The enzyme PDE-5 breaks down cyclic guanosine monophosphate (cGMP), a significant signalling molecule in smooth muscle relaxation and vasodilation [59]. Diabetes often leads to increased PDE-5 activity, which in turn reduces cGMP levels and impacts vascular function. The study samples were found to repress PDE-5 activity, which in turn could allow cGMP to accumulate and promote vasodilation. This activity may enhance blood flow, insulin secretion, and overall pancreatic function [63].

In diabetes research, there is scientific interest in the mechanism of purinergic enzymes on the well-being of the pancreas of a diabetic subject and its interaction

with PHT administration. Purinergic enzymes (ADA, ATPase, and 5'-nucleotidase) play a crucial role in regulating physiological processes, such as insulin secretion [76, 77]. Pancreatic beta cells, the insulin-producing cells, become dysfunctional in the presence of high activity of purinergic enzymes [78]. PHT has been investigated as a potential medicinal agent due to its anti-inflammatory and antioxidant properties, as well as its ability to alter pancreatic purinergic enzyme activity in diabetic rats [79, 80]. The bioactive substances in the tea could interact with purinergic receptors to affect ATP release and, subsequently, insulin secretion [78]. For instance, certain elements found in tea, such as flavonoids and polyphenols, may promote the release of ATP from beta cells, thereby enhancing insulin production [7]. These substances can also impede ADA action, preventing the breakdown of adenosine (essential molecules in the insulin secretion process) [78, 80].

The revealing connection between diabetes and hypertension, as well as its relationship to oxidative stress, is among the objectives of this study. This study reinforced the previous findings on the effect of oxidative stress on the pathophysiology of diabetes/hypertension comorbidity. Oxidative stress is characterised by decreased activity of antioxidant enzymes such as SOD, CAT, GPx, and GST [81, 82]. Plant materials high in polyphenols are a significant source of antioxidants and can reduce oxidative stress [83, 84]. Similar to earlier research, the improved antioxidant activity of these molecules suggests a reduction in oxidative stress and may be related to the presence of phytochemicals, particularly phenolic compounds [85, 86]. According to the observed outcomes, using PHT infused for 15 min had more excellent antioxidant effects and might fortify the antioxidant defence system, shielding the pancreas tissue from oxidative damage.

Hypertension often coexists with diabetes, and both conditions can exacerbate each other's complications [87–89]. PHT's potential to address diabetes and obesity is a promising natural remedy for DM+HYP [90]. The effect of PHT on the NRF2 gene (Fig. 5) in the pancreatic tissue of diabetes-hypertensive rats is an intriguing component of the study. An essential aspect of cellular defence systems, particularly in response to oxidative stress, is played by the transcription factor NRF2 [91, 92]. Diabetes-hypertension worsens because of oxidative stress. According to research, PHT may alter the expression of the NRF2 gene, enhance antioxidant defences, and possibly reduce the harm caused by oxidative stress [93, 94]. The pancreas tissue of the treated rats exhibited an increase in the NRF2 gene expression. These findings can be related to the studies of Uddandrao et al. [96] and Aslam et al. [97] on herbal formulations, where it was observed that oxidative stress resulted in DM and its

complications, leading to the downregulation of NRF2, while depending on HO-1 [98]. Increased production and activity of antioxidant enzymes, including SOD and catalase, could be the aftermath of this upregulation [95]. As a result, the rats showed improved blood pressure and glycemic management. This could be another mechanism by which Polyherbal tea can exercise its therapeutic effects. Polyherbal tea may aid in preserving beta cell function and insulin secretion, which are crucial for maintaining glucose homeostasis by strengthening the pancreas's antioxidant defences.

6 Conclusion

This research provides information on the ability of polyherbal tea to reduce fasting blood glucose and systolic and diastolic blood pressure to justify its administration against co-morbidity. The potential of polyherbal tea to upregulate the nuclear factor 2 (NRF2) and improve cellular antioxidants describes its capacity. Although the limitations of the study prevented us from arguing via western blotting or histological analysis of the animal tissues, the application of polyherbal extracts as a means for managing diabetes and hypertension might require further investigation, which we are currently exploring in our lab.

7 Limitations of the Study

This study was unable to monitor the downstream effectors, such as HO-1 and NQO1, as well as the histology of the pancreas, liver and kidney, which could have strengthened the findings of this study. This is one of the limitations of our findings.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s44424-025-00022-2>.

Below is the link to the electronic supplementary material. Supplementary file1 (DOCX 141 KB)

Author Contributions

I.S.O -Conceptualization, Second Draft. O.R.O -Methodology, Animal Handling, Assay, First draft. O.B.O -Methodology, Molecular study. A.O.A—Third draft review and G.O -Conceptualization, Resources.

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Data Availability

Data will be available on reasonable request.

Declarations

Conflict of Interest

The authors declare no competing interests.

Ethical Approval

The Federal University of Technology, Akure's Ethical Committee, under the guidance of the National Institutes of Health, granted permission for the use of albino rats in this research. The approved study was assigned the reference number FUTA/SOS/1411, after which it underwent a strict review and adherence to guidelines for the handling of animals.

Clinical trial number

Not applicable.

Author details

¹Department of Medical Biochemistry, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria. ²Functional Foods, Nutraceuticals and Phytomedicine Laboratory Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria.

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