Antioxidant Status of Liver and Kidney Homogenates from Hyperglycemic Rats Administered with Single and Combinatorial Herbal Formulations

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ABSTRACT

Aims: The present study ascertained the comparative capacities of single and combinatorial herbal formulations of *Acanthus montanus*, *Emilia coccinea*, *Hibiscus rosasinensis* and *Asystasia gangetica* to exert glycemic control and ameliorate oxidative stress in renal and hepatic tissues of alloxan-induced hyperglycemic rats (HyGR) following 30 days treatment. **Materials and Methods:** Phytochemical composition of the herbal extracts and fasting plasma glucose concentration (FPGC) of the rats were measured by standard methods. The post mitochondrial supernatant fractions (PMSF) of renal and hepatic tissues homogenates were measured for activity levels of glutathione peroxidase (GPOx), superoxide dismutase (SOD) and catalase (CAT) as well as glutathione (GSH) concentration. **Results:** Comparatively, flavonoids were the most abundant phytochemical present in the four herbal extracts. FPGC of treated HyGR was within the range of 66.30 ± 0.71-133.00 ± 0.63 mg/dL as against the untreated hyperglycemic rat group=368.20 ± 0.72 mg/dL (p<0.05). At the end of the experimental time, treated HyGR liver SOD activities were lower than that of normal group (p<0.05). Liver and kidney GPOx activities of HyGR were 2.36 folds and 3.93 folds lower than the normal rats. Furthermore, liver and kidney CAT activities of the treated HyGR were significantly (p<0.05) lower than the normal rats, whereas liver and kidney GSH/GSSG ratios were significantly (p<0.05) increased when compared with treated HyGR. **Conclusion:** Combinatorial herbal formulations exerted greater glycemic control than the single herbal formulations and caused a sparing effect on renal and hepatic antioxidants against reactive oxygen species.

Key words: Antioxidant, Hepatic, Hyperglycemia, Phytochemicals, Renal.

INTRODUCTION

Hyperglycemia, which is one of the prominent defining features of diabetes mellitus, has been implicated in promoting overwhelming levels of reactive oxygen and nitrogen species (RONS) and protein glycation.[1] Hyperglycemia mediated generation and accumulation of RONS may occur through one or combinations of the following molecular events: distortions of glycolytic pathways, intercellular activation of sorbitol (polyol) pathways, auto-oxidation of glucose, non-enzymatic proteins and amino acids glycation.[1] Antioxidant systems prevent cellular damage from the adverse effects of xenobiotics, carcinogens and cytotoxic activities of RONS. Several reports showed that there are several biologically derived compounds with antioxidant functions.[2] Glutathione peroxidases (GPOx; EC 1.11.1.19) are ubiquitous selenium-containing peroxidases that catalyze the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH as co-factor. GPOx activity, among other functions, protects mammalian cells against oxidative damage. There are at least five GPOx isoenzymes found in mammals with varying levels of activity of each isoform

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depending on the tissue type. Superoxide dismutase (SOD; EC 1.15.1.1) is metallo-enzyme that defines the basis for their classification; Mn-SOD,[3]Cu, Zn-SOD[2] and Ni–SOD.[4] SOD activity neutralizes superoxide by transforming oxygen derived reactive species to peroxide, which can in turn be eliminated by the actions of catalase or GPOx activities. Catalase (CAT; EC 1.11.1.6) is a tetrameric haemin-enzyme consisting of four identical tetrahedrally arranged subunits of ≈ 60 kDa. CAT activity prevents H_2O_2-induced cellular damage by converting H_2O_2 to H_2O and O_2. To a large extent, the thiol-redox status of the cell is defined by cellular levels of GSH. In addition, the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio (GSH: GSSG) is an important biomarker of redox environment in biologic systems.

In modern medicine and traditional systems, medicinal plants have continued to provide valuable therapeutic agents for the treatment and management of diseases and disorders. Moreover, in spite of the higher acceptability level of orthodox anti-diabetic therapeutics, the use several plants as herbal remedies by traditional healers for the treatment of DM is still very common especially in Africa and Asia continents.[5,6] Traditional herbs are commonly used in the form multi-herb recipe, which is believed to possess better pharmacological efficacy than the individual composite herb extracts. In that regard, the present study ascertained the comparative capacities of single and combinatorial herbal formulations of four medicinal leaf extracts of *Acanthus montanus*, *Emilia coccinea*, *Hibiscus rosasinensis* and *Asystasia gangetica* to exert glycemic control in alloxan-induced hyperglycemic rats (HyGR). In addition, hyperglycemia causes oxidative organ damage, mediated by autoxidation of glucose. Cellular levels of the antioxidant system could serve as a reliable biomarker to establishing the pathogenesis and progression of DM especially with respect to tissue damage. Therefore, studies were carried out to ascertain levels of renal and hepatic tissues derived primary oxidative stress indicators (GPOx, SOD, CAT activities and GSH/GSSG ratio) and their correlations, following the administration of the herbal formulations. The outcome of the present investigations will serve to give insights into the capacities of the various herbal formulations to ameliorate hyperglycemia and tissue derived oxidative stress in DM.

MATERIALS AND METHODS

Collection and preparation of herbal samples

Fresh leaves of *Acanthus montanus* (Nees) T. Anderson (ACMO), *Emilia coccinea* (SIMS) G. Don (EMCO) and *Hibiscus rosasinensis* L. (HIRO) were collected from uncultivated lands in Umuamacha Ayaba Umaeze, Osisioma Ngwa LGA, Abia State, Nigeria, whereas fresh leaves of *Asystasiagangetica* L. T. Anderson (ASGA) were collected from Ubowuala, Emekuku, Owerri North Local Government Area, Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe at a School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri. All the leaves were collected between the months of July and August, 2009.

The leaves of individual plants were washed with continuous flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature (24 ± 5°C). A 500 g part of each herbal samples were weighted using a triple beam balance (OHAU 750-50: Burlington, NC, USA) and dried in an oven (WTC BINDER, 7200 Tuttlingen, Germany) at 60°C until a constant weight was achieved. The dried leaves were packaged in dark polyethylene bags and kept in cold room (7 ± 3°C) for 24 h before pulverization. Next, the separate dried leaves were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA), after which the ground samples were stored in air-tight plastic bottles with screw caps pending extraction.

Extraction of herbal samples

Portions of 40 g of each pulverized dried samples of *A. montanus*, *E. coccinea*, *H. rosasinensis* and *A. gangetica* were subjected to repeated soxhlet extraction cycles for 2 h using 96% C_2H_5OH (BDH, U.K) as solvent to obtain a final volume of 500 mL of each herbal extracts. These volumes of the extracts were concentrated and recovered in a rotary evaporator for 12 h at 60°C under reduced pressure. The extracts were dried in a desiccator for 24 h, wrapped in aluminum foil and stored in air-tight plastic bottles with screw caps at ≤ 4°C. The yields were calculated to be as follows: *A. montanus* = 16.35% (*w*/*w*), *E. coccinea* = 17.99% (*w*/*w*), *H. rosasinensis* = 17.23% (*w*/*w*) and *A. gangetica* = 16.69% (*w*/*w*). The separate extracts were reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCI, 17.0 g $Na_2HPO_4.2H_2O$ and 2.43 g $NaH_2PO_4.2H_2O$), before appropriated doses were administered to the experimental animals. Portions of the individual extracts were also measured for phytochemical contents.

Phytochemical composition of herbal extracts

Flavonoids content was measured by the methods.[7] The concentrations of alkaloids, tannins and saponin of the herbal extracts were measured by the methods.[8]

Experimental animals

Male albino (Wistar) rats weighing between 150-160 g were maintained at room temperatures of 24 ± 5°C, 30–55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and standard commercial feed (SCF) (Ewu Feed Mill, Edo State, Nigeria) *ad libitum* for 2 weeks acclimatization period. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Induction of diabetes/experimental design

Hypoglycemia was induced in the rats by single intra-peritoneal (i.p) injection of 90 mg/kg *bw* of alloxan monohydrate (Sigma, St. Louis, USA) in PBS solution (pH=7.4). The animals with fasting plasma glucose concentration (FPGC) > 110 mg/dL for 5 consecutive days were considered diabetic and selected for the study. A total of 102 male Wistar rats were allotted into seventeen (17) groups of six (6) rats each. The animals were deprived of food and water for additional 16 h before commencement of treatment as described elsewhere.[9] The animal groups were designated on the basis of treatments received at regular intervals of 2 days for 30 days. Herbal treatments of the HyGR were defined as single herbal formulations (SHF): (HrACMO, HrASGA, HrEMCO and HrHIRO), double herbal formulations (DHF): (HrAGAM, HrAGEC, HrAGHR, HrAMEC, HrAMHR and HrECHR), triple herbal formulations (THF): (HrAGEH, HrAMAE, HrAMAH and HrAMEH) and quadruple herbal formulation (QHF): (HrAAEH).

- NORM: Normal rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- DIAB: HyGR received SCF + water *ad libitum* + 1.0 mL/kg of PBS.
- HrACMO: HyGR received SCF + water *ad libitum* + *A. montanus* (20 mg/kg in PBS; i.p.).
- HrASGA: HyGR received SCF + water *ad libitum* + *A. gangetica* (20 mg/kg in PBS; i.p.).
- HrEMCO: HyGR received SCF + water *ad libitum* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrHIRO: HyGR received SCF + water *ad libitum* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAGAM: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *A. montanus* (20 mg/kg in PBS; i.p.).
- HrAGEC: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAGHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. gangetica* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMEC: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAMHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *A. montanus* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrECHR: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1 *w/w*) of *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAGEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. gangetica* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMAE: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* (20 mg/kg in PBS; i.p.).
- HrAMAH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAMEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1 *w/w*) of *A. montanus* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).
- HrAAEH: HyGR received SCF + water *ad libitum* + combined dose (ratio: 1:1:1:1 *w/w*) of *A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.).

At the end of treatment, the animals were fasted for 12 h[5] before their blood samples and organ homogenates were measured for the various biochemical parameters.

Fasting plasma glucose concentration

Blood samples (2.0 mL) were drawn from the orbital sinus on the 30th day and measured for FPGC. Determination of FPGC was by the glucose oxidase method according

Table 1: Fasting plasma glucose concentrations of normal, diabetic and treated rats

Group	FPGC (mg/dL)
NORM	86.30 ± 0.15^{n}
DIAB	368.20 ± 0.72^{a}
HrACMO	133.00 ± 0.63^{b}
HrASGA	105.70 ± 0.71^{e,f,g,h}
HrEMCO	118.00 ± 0.92^{c,d}
HrHIRO	112.50 ± 0.70^{d,e}
HrAGAM	66.30 ± 0.71^{q}
HrAGEC	81.00 ± 0.39^{n,o,p}
HrAGHR	112.50 ± 0.42^{d,e,f}
HrAMEC	97.20 ± 0.51^{l,m}
HrAMHR	105.00 ± 0.62^{e,f,g,h,i}
HrECHR	103.50 ± 0.79^{f,g,h,i,j}
HrAGEH	103.00 ± 0.81^{f,g,h,i,j,k}
HrAMAE	84.70 ± 0.51^{n,o}
HrAMAH	124.30 ± 0.92^{c}
HrAMEH	112.00 ± 0.71^{d,e,f,g}
HrAAEH	97.30 ± 0.85^{l}

The mean (X) ± S.D of six (n=6) determinations. Means in the column with the same letter are not significantly different at p>0.05 according to LSD. FPGC>110 mg/dL = hyperglycemia

to Randox® kit manufacturer's procedure (Randox® Laboratories Ltd. Ardmore, United Kingdom).

Preparation of renal and hepatic organs homogenates

The rats were anaesthetized in CCl_4 chamber and dissected. The kidneys and liver were quickly excised and placed on a blotting paper to remove blood and rinsed in 1.15% KCl solution to remove residual hemoglobin.[10] The two organs were stored in 10%H_2CO and preserved at temperature below -8 °C before analyses. Preparation of the organ homogenates was according to the methods10 with minor modifications by Chikezie and Uwakwe,[11] Each organ was homogenized using a Teflon homogenizer in aqueous K_2PO_4/$KHPO_4$ buffer (0.1 M; pH=7.4); in 4:1 volume of buffer to organ weight. Next, the crude homogenate was centrifuged at 10,000 × g for 20 min at 4°C to obtain the post mitochondrial supernatant fraction (PMSF). The PMSF was finally stored at -8°C before used for analyses. The PMSF was measured for GPOx, SOD and CAT activities. Protein concentrations of PMSF of hepatic and renal homogenates were assayed by the method[12] using bovine serum albumin (Sigma-Aldrich, St. Louis, USA) as a standard. Also, the PMSF was measured for glutathione (GSH) concentration.

Glutathione peroxidase activity

GPOx activity was measured by the method reported by Chikezie and Uwakwe,[11]

Superoxide dismutase activity

SOD activity was measured according to the methods of Kono,[13]

Catalase activity

Measurement of PMSF CAT activity was according to the methods reported by Chikezie and Uwakwe,[11]

Reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio

Level of GSH in organ homogenate was determined according to modified methods of Chikezie and Uwakwe,[11] The GSSG concentration of organ homogenate was measured using Bioxytech-412 kits according to manufacturer's procedure (Oxis International Inc., Foster City, CA, USA).

Statistical analysis

The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 versions, (2006). Correlation coefficients and trend lines were determined using Excel Software (Microsoft, 2010 version).

RESULTS

At the end of the experimental time of 30 days, FPGC of DIAB group was 4.3 folds > NORM group (Table 1). Specifically, HrACMO, HrHIRO, HrAGHR and HrAMEH groups exhibited elevated FBGC compared with the NORM group (p<0.05) and were considered to be hyperglycemic after the 30-day treatment. Conversely, FPGC of HrASGA, HrAMEC, HrAMHR, HrECHR, HrAGEH and HrAAEH groups were significantly (p<0.05) higher than the NORM group but not considered to be hyperglycemic after the 30-day treatment.

Figure 1 showed that flavonoids were comparatively the most abundant phytochemical present in the four leaf extracts, which was in the order: AMCO > ASGA > HIRO > EMCO. The concentration of alkaloids in HIRO was relatively low compared with other three leaf extracts.

Liver SOD (LSOD) activity of NORM group was significantly (p<0.05) higher than the corresponding kidney SOD (KSOD) activity (Figure 2). Likewise, in the DIAB

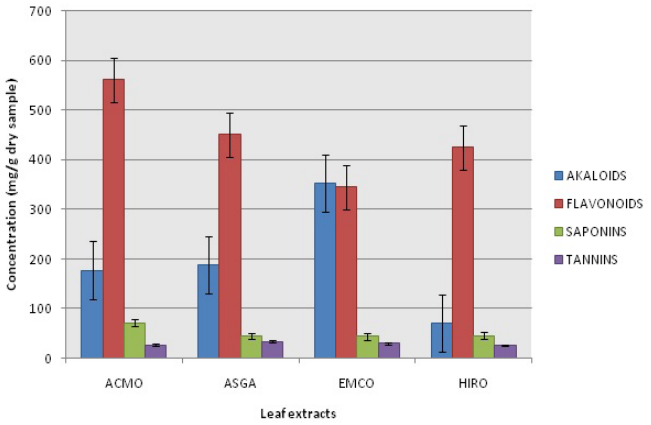

Figure 1: Some phytochemical contents of *A. montanus*, *A. gangetica*, *E. coccinea* and *H. rosasinensis* leaf extracts.

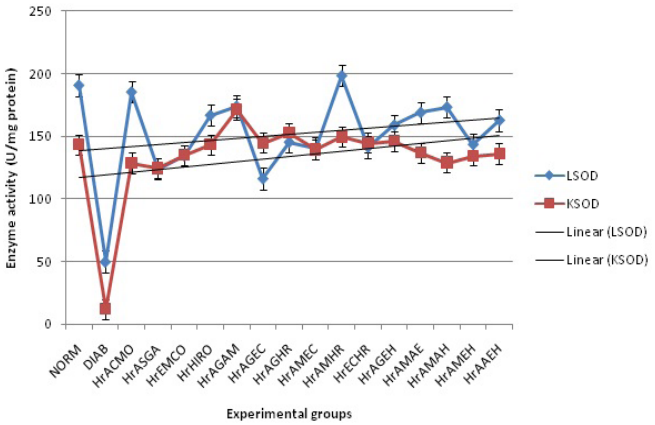

Figure 2: Liver and kidney superoxide dismutase activities of experimental rats.

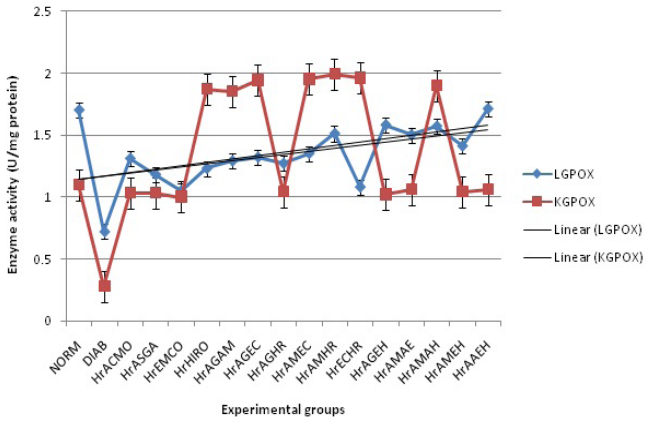

Figure 3: Liver and kidney glutathione peroxidase activities of experimental rats

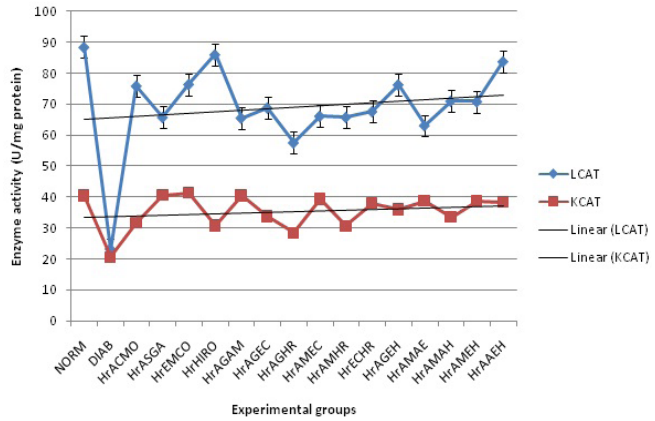

Figure 4: Liver and kidney catalase activities of experimental rats.

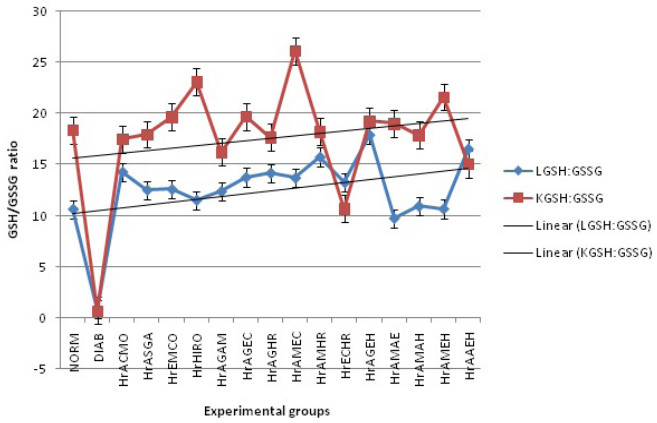

Figure 5: Liver and kidney ratio of GSH to GSSG concentrations of experimental rats.

group, LSOD activity was higher than KSOD activity; $p>0.05$. Also, Figure 2 showed that the levels of LSOD and KSOD activities of the DIAB group were in 3.82 and 12.01 folds lower than the NORM group, relatively.

However, KSOD activities of other treated HyGR (HrAGEC, HrAMAH, HrECHR and HrAGEH) were higher than the NORM group, but with no significant difference ($p>0.05$). Generally, the treated HyGR exhibited increased levels of LSOD and KSOD activities compared with the DIAB group. For instance, HrAMHR group showed 3.97 folds improvement in LSOD activity compared with DIAB group. LSOD and KSOD activities of the treated HyGR showed a very weak positive correlation ($r=0.197011749$).

Liver GPOx (LGPOx) activity of the NORM group was not significantly ($p>0.05$) different from the corresponding

kidney GPOx (KGPOx) activity. LGPOx and KGPOx activities of DIAB group were 2.36 folds and 3.93 folds lower than the NORM group (Figure 3).

Liver CAT (LCAT) activity was 2.19 folds > kidney CAT (KCAT) activity in NORM group (Figure 4). $DIAB_{LCAT}$ activity and $DIAB_{KCAT}$ activity represented 73.93% and 48.60% reduction in CAT activity compared with NORM group; $p<0.05$. Comparative analyses showed that $DIAB_{LCAT}$ activity was not significantly ($p>0.05$) different from $DIAB_{KCAT}$ activity.

Figure 5 showed that most liver and kidney GSH/GSSG ratios were significantly ($p<0.05$) increased when compared with NORM group, following the administration of the herbal formulations. LGSH: GSSG and KGSH: GSSG) of treated hyperglycemic rat groups showed a very weak negative correlation (r=-0.192889137).

DISCUSSION

Previous studies showed that alkaloids and flavonoids from natural products, including plant materials are effective anti-diabetic agents.[14] In another study, the potency of core structures of flavonoids precursors to act as anti-hyperglycemic agent was demonstrated *in vivo* in HyGR.[15] A comprehensive review on natural approach to the treatment of diabetes[16] further confirmed that flavonoids are among the listed anti-diabetic compounds, which exert their hypoglycemic properties via extra pancreatic mechanism of α-glucosidase modulation. Expectedly, the relatively high flavonoids contents of the four medicinal plants (ACMO, ASGA, EMCO and HIRO) contributed to the capabilities of the herbal formulations to exert glycemic control in the experimental rats. Likewise, the relatively high abundance of alkaloids, especially in ACMO, ASGA and EMCO extracts, may have acted as a hypoglycemic agent as previously reported.[17] Therefore, combinatorial formulations of the four medicinal plant extracts promoted anti-hyperglycemicsynergy amongst the various bioactive principles, and thereby potentiated the glycemic control indices of the individual leaf extracts in conformity to previous reports[6] Also, previous investigations have demonstrated that combining ginger extracts' with other constituents, particularly 6-gingerol, caused significant augmentation of anti-proliferative activity of the extracts. [17] Paradoxically, studies have equally shown that antagonistic inter-phytochemicals interactions in medicinal plant extracts could, attenuate the therapeutic potentials of the individual bioactive principles.[18]

Saponins and tannins are bioactive principles of medicinal and toxicity importance and their capabilities to exert glycemic control have been experimentally established.[19-21] Kunyanga *et al*,[19] reported that condensed tannin extracts of raw and processed indigenous food ingredients from Kenya exhibited promising anti-diabetic effects; possessing potential α-amylase and α-glucosidase inhibition activities within the range of 23% to 44% and 58% to 88%, respectively. Another study,[20] implicated the saponins as the anti-diabetic principles of seed extract of *Entada phaseoloides* L. They further posited that the therapeutic effect of saponins was facilitated by repression of chronic inflammation response pathways of pancreatic islets that was mediated by its inhibitory actions on interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) by the seed extracts in a dose dependent manner. Histopathological examination of pancreatic tissues of streptozotocin-induced diabetic rats (S-IDR)[21] showed evidence of considerable quantitative increase in β-cells by 75% when treated with saponins. The present study showed that the saponins and tannins are present in approximately equal proportions in extracts of ACMO, ASGA, EMCO and HIRO that may have contributed, in part, to the anti-hyperglycemic potentials of the experimental plant extracts.

Generally, the level of enzyme activity in biologic systems could serve as a reliable tool in ascertaining health and pathologic conditions.[2] Also, in the event of exposure of biological systems to noxious chemical agents and other environmental insults, previous researchers have applied levels of activity of diverse antioxidant enzymes and low molecular weight antioxidants (LMWA) as basis for ascertaining tissue oxidative stress status[2,22] Findings from the present study showed that HyGR exhibited alterations in tissue antioxidant enzymes (SOD, GPOx and CAT) activities and GSH/GSSG ratio, of which the cellular levels of these oxidative stress indicators were diagnostic of oxidative tissue injury.

Decreased SOD activity of untreated HyGR as reported here conformed to previous observations elsewhere.[1,23] Particularly, DiNaso *et al.,*[1] noted that decreased SOD activity in DM is connected with non-enzymatic glycation of intracellular SOD at the lysine residues located in the heparin-binding domain, which indirectly altered extracellular SOD activity and functionality. Another study,[24] posited that decreased Cu-Zn SOD activity observed in DM was sequel to the inactivation of the enzyme as a result of absence or failure to metabolize hydrogen peroxide via GPOx pathway. The decreased levels of renal and hepatic SOD activity of HyGR showed evidence of restoration of the enzyme activity that were

comparable to NORM rats following the administration of the various experimental herbal formulations (Figure 2). The present findings are comparable with previous report[25] in which they noted that glibenclamide directly increased renal and hepatic CAT and SOD activities of S-IDR. Reports have shown that ethanolic extract of *Sphaeranthus indicus* exhibited protective effect against lipid peroxidation and normalized repressed SOD, CAT, glutathione *S*-transferase activities in gentamicin induced nephrotoxic rats.[22] In the same vein, previous reports showed that S-IDR treated with exogenous SOD and GSH ratio exhibited improved antioxidant enzymes activities[1,24] which implied amelioration of tissue oxidative stress.

Depressed renal and hepatic GPOx and CAT activities were associated with the diabetic state as reported here (Figures 3 and 4). Specifically, previous studies[26] had noted that CAT deficiency accelerated diabetic nephropathy, which was mediated by peroxisomal dysfunction. However, cod liver oil intake engendered significant increase in both GPOx and CAT activities in aorta, heart, and liver of S-IDR,[27] thereby alleviated oxidative stress induced tissue injuries. Similarly, the studies[23] revealed that dietary ginger improved renal and hepatic GPOx and CAT activities, alongside other antioxidant enzymes and oxidative stress indicators of S-IDR. They further noted that treatment of diabetic rats with ginger for 30 days caused a therapeutic protective effect by reducing oxidative stress, hepatic and renal damage. Also, using experimental female Wistar rats, several natural products obtained from wide varieties of medicinal plants reversed low levels of tissue SOD, CAT, GPOx and GST activities following exposure to cadmium. They further suggested that their therapeutic actions were probably connected with the synergistic effects of the bioactive principles of the natural products. The present study has equally shown substantial improvements in depressed renal and hepatic GPOx and CAT activities in HyGR following the administration of combinatorial herbal formulations of the four medicinal leaf extracts.

The physiochemical roles of GSH is indispensible in a multitude of cellular processes. Therefore, disturbances in GSH homeostasis are associated with the etiology and/or progression of a number of human pathology. Furthermore, the redox state of the GSH/glutathione disulfide couple (GSH/GSSG) is an important biologic indicator and regulator of signals of and metabolic pathways in normal cell systems as indicators of level of well as oxidative stress and glucose metabolism in type 2 DM. Accordingly, the results of the present investigations have shown that GSH/GSSG ratio is an important and reliable biomarker for hyperglycemia and for ascertaining the efficacy of herbal remedies for alleviation of hyperglycemia and amelioration of tissue oxidative stress. The present findings corroborated the reports of El-Missiry and El Gindy,[28] in which they noted that daily oral administration of *Eruca sativa* seeds oil for 2 weeks stimulated and increased hepatic GSH production of alloxan-treated rats.Zitka *et al*.,[29] used the redox status expressed as GSH:GSSG as a biomarker for ascertaining the level of oxidative stress in paediatric tumor patients, which was similar to earlier reported Murakam *et al*.,[30] on impairment of glutathione metabolism in erythrocytes of patients suffering from DM.

CONCLUSION

From the present investigations, improvement in renal and hepatic antioxidant enzymes activities and GSH: GSSG ratio of the treated HyGR suggest that the experimental herbal formulations caused a sparing effect on renal and hepatic antioxidant system against reactive oxygen species. Additionally, the combinatorial herbal formulations exerted greater glycemic control than the single herbal formulations.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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REFERENCES

1. Di Naso FC, Dias AS, Porawski M, Marroni NAP. Exogenous superoxide dismutase: action on liver oxidative stress in animals with streptozotocin-induced diabetes. Exp. Diabetes Res. 2011; 2011 Exp Diabetes Res. 2011; 2011: 754132. Published online Mar 13, 2011. doi: 10.1155/2011/754132: 754132. 7 pages. doi: 10.1155/2011/754132
2. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol. 2002; 30(6): 620-50.
3. Guan Y, Hickey MJ, Borgstahl GE, Hallewell RA, Lepock JR, O'Connor D, *et al*. Crystal structure of Y34F mutant human mitochondrial manganese superoxide dismutase and the functional role of tyrosine 34. Biochem 1998; 37(14): 4722–30.
4. Young H, Kim EJ, Roe JH, Hah YC, Kang SO. A novel nickel-containing superoxide dismutase from *Streptomyces spp*. Biochem J. Sep 15, 1996; 318 (Pt 3): 889–896.
5. El-Demerdash FM, Yousef MI, Abou El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats.

Food Chem. Toxicol. 2005; 43(1): 57–63.

6. Wang F, Zhao S, Li F, Zhang B, Qu Y, Sun T, *et al*. Investigation of antioxidant interactions between *Radix Astragali* and *Cimici fugafoetida* **a**nd identification of synergistic antioxidant compounds. Plos One. 2014; 9(1): e87221. doi:10.1371/journal.pone.00872.
7. Boham AB, Kocipai AC. Flavonoid and condensed tannins from leaves of Hawaiian *Vaccininum raticulum* and *V. calycinium***.** Pacific Sci. 1994; 48 Pacific Science, 48: 458-463.
8. Belonwu DC, Ibegbulem CO, Nwokocha MN, Chikezie PC. Some phytochemicals and hydrophilic vitamins of *Anacardium occidentale.* Res. J. Phytochem. 2014; 8(3): 78-91.
9. Ibegbulem CO, Chikezie PC. Hypoglycemic properties of ethanolic extracts of *Gongronema latifolium***,** *Aloe perryi, Viscum album* and *Allium sativum* administered to alloxan-induced diabetic albino rats (Rattusnorvegicus). Pharmacog. Commun. 2013; 3(2): 12-6.
10. Adekunle AS, Adedeji AL, Oyewo EO, Adedosu OT, Omotoso AT. Hyperlipidemia induced by atherogenic diet enhanced oxidative stress in the kidney and inflammatory responses: an *in-vivo* study. Asian J. Nat. Appl. Sci. 2013; 2(1): 82-93.
11. Chikezie PC, Uwakwe AA. Activities of three erythrocyte enzymes of hyperglycemic rats (*Rattus norvegicus*) treated with *Allium sativa* **e**xtract. Journal of Diabetes & Metabolic Disorders 2014, 13:50. http://www.jdmdonline.com/content/13/1/50. 2014; 13 50, 9. doi: 10.1186/2251-6581-13-50.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951; 13(1): 265–75.
13. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 1978; 186(1): 189–95.
14. Chen F, Xiong H, Wang J, Ding X, Shu G, Mei Z. Antidiabetic effect of total flavonoids from *Sanguisdraxonis* in type 2 diabetic rats. J. Ethnopharmacol. 2013; 149(3): 729-36.
15. Najafian M, Ebrahim-Habibi A, Yaghmaei P, Parivar K, Larijani B. Core structure of flavonoids precursor as an anti-hyperglycemic and anti-hyperlipidemic agent: An *in vivo* study in rats. Acta. Biochim. Pol. 2010; 57(4): 553-60.
16. Kumar S, Narwal S, Kumar V, Prakash O. α-Glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacog. Rev. 2011; 5(9): 19-29.
17. Brahmbhatt M, Gundala SR, Asif G, Shamsi SA, Aneja R. Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. Nutr. Cancer. 2013; 65(2): 263-72.
18. Yao Y, Luong TN, Lepik M, Aftab N, Fong VH, Vieira A. Synergism of antioxidant phytochemicals: comparisons among purified polyphenols and dietary-plant extracts. ISHS Acta Horticulturae 939: XXVIII International Horticultural Congress on Science and Horticulture for People (IHCSHP): International Symposium on Emerging Health Topics in Fruits and Vegetables; 2010.
19. Kunyanga CN, Imungi JK, Okoth M, Momanyi C, Biesalski HK, Vadivel V. Antioxidant and antidiabetic properties of condensed tannins in acetonic extract of selected raw and processed indigenous food ingredients from Kenya. J. Food Sci. 2011; 76(4): C560-7.
20. Zheng T, Shu G, Yang Z, Mo S, Zhao Y, Mei Z. Antidiabetic effect of total saponins from *Entada phaseoloides* (L.) Merr in type 2 diabetic rats. J. Ethnopharmacol. 2012; 139(3): 814-21.
21. Koneri RB, Samaddar S, Ramaiah CT. Antidiabetic activity of a triterpenoid saponin isolated from *Momordica cymbalaria* Fenzl. Indian J. Exp. Biol. 2014; 52(1): 46-52.
22. Mathew JE, Mantri A, Vachala SD, Srinivasan KK, Movaliya V. Effect of *Sphaeranthus indicus* ethanol extract on tissue antioxidant activity in gentamicin induced nephrotoxic rats. Herba. Polonica. 2009; 55(4): 86-95.
23. Shanmugam KR, Mallikarjuna K, Nishanth K, Kuo CH, Reddy KS. Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. Food Chem. 2011; 124(4): 1436–42.
24. Loven D, Sched H, Wilson H, Daabees TT, Stegink LD, Diekus M, Oberley L. Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozocin-induced diabetes. Diabetes 1986; 35(5): 503-7.
25. Elmalí E, Altan N, Bukan N. Effect of the sulphonylurea glibenclamide on liver and kidney antioxidant enzymes in streptozocin-induced diabetic rats. Drugs R & D. 2004; 5(4): 203-8.
26. Hwang I, Lee J, Huh JY, Park J, Lee HB, Ho Y-S, Ha H. Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction. Diabetes 2012; 61(3): 728-38.
27. Hünkar T, Aktan F, Ceylan A, Karasu C. Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. Cell Biochem. Funct. 2002; 20(4): 297-302.
28. El-Missiry MA, El Gindy AM. Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of Eruca sativa seeds. Ann. Nutri. Metab. 2000; 44(3): 97–100.
29. Zitka O, Skalickova S, Gumulec J, Masarik M, Adam V, Hubalek J, et al. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumor patients. Oncol. Lett. 2012; 4(6): 1247-53.
30. Murakami K, Takahito K, Ohtsuka Y, Fujiwara Y, Shimada M, Kawakami Y. Impairment of glutathione metabolism in erythrocytes from patients with diabetes mellitus. Metab. 1989; 38(8): 753–8