Serum Lactate Dehydrogenase Activity and Visceral Organs and Body Weights of Diabetic Rats Administered Single and Combinatorial Herbal Formulations

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ABSTRACT

Background and Aim: The present study evaluated serum lactate dehydrogenase (LDH) activity. Visceral organs and body weights were used as measures of the capacities of single and combinatorial herbal formulations of Acanthus montanus, Asystasia gangetica, Gongronema latifolium and Solanum melongenas to ameliorate systemic toxicity, visceral organs inflammation or necrosis and body tissues wasting in alloxan-induced diabetes mellitus (DM) rats. Materials and Methods: Alloxan-induced DM rats with fasting plasma glucose concentration (FPGC) > 5.71 mmol/L for 5 consecutive days were selected for the study. A total of 102 male Wistar rats were divided into seventeen (17) groups of six (6) rats each. Serum LDH activity and body weights and weights of visceral organs and were measured using standard methods. Results: Serum LDH activities of herbal treated rat groups varied within a relatively narrow range of 549.9 ± 12.10 – 500.6 ± 12.02 IU/L and were significantly lower (p < 0.05) than the untreated DM rat group. The body weights of the experimental rat groups after herbal treatment were significantly higher (p < 0.05) than their corresponding weights before herbal treatment. The ratios of liver weights to body weights were within the range of 0.0293 ± 1.4 x 10⁻³ – 0.0597 ± 2.3 x 10⁻³. The ratio of kidney weight to body weight of untreated DM rat group was 1.64 fold higher than that of normal rat group (p > 0.05). Conclusion: Overall, 200 mg/kg body weight double herbal formulations of A. gangetica + A. montanus and A. gangetica + G. latifolium offered the greatest therapeutic benefits to alloxan-induced DM rats, with respect to all diagnostic parameters considered in the present study.

Key words: Body weights, Diabetes mellitus, Herbal formulations, Lactate dehydrogenase, Visceral organs.

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INTRODUCTION

Lactate dehydrogenase (LDH; EC 1.1.1.27) is an oxido-reductase and cytoplasmic enzyme that catalyzes the reversible reaction: L-lactate + NAD⁺ ↔ pyruvate + NADH. The LDH enzyme is a tetrameric protein and is composed of five possible isoenzymes, designated as LDH₁, LDH₂, LDH₃, LDH₄, and LDH₅. Heart, kidney, brain and erythrocytes are composed of one or two of the five possible isoenzymes of LDH. The erythrocyte cells have the highest proportion of LDH₁, and LDH₅, whereas the liver and skeletal muscle has the highest percentage of LDH₃. Clinical applications of LDH activity involve quantification of one or more specific serum isoenzymes. Accordingly, mild elevation in serum LDH activity has been reported in cases of haemolytic anemia, muscular dystrophy, pulmonary infarction, hepatitis, nephritic syndrome and cirrhosis. Diabetes mellitus (DM) is multi-faceted endocrine disorder associated with visceral organs necrosis, inflammation and damage, with accompanying general body tissues wasting and systemic toxicity outcomes. Alloxan systemic toxicity arises following the metabolism of alloxan to dialuric acid, which undergoes redox cycling to generate overwhelming levels of superoxide radicals. Dismutation of superoxide radicals yields hydrogen peroxide, whereas Fenton reaction pathways of superoxide radicals generate hydroxyl radicals. The elevated RONS levels are deleterious to organelles and enzymes, and thereby promote injuries to body tissues. The phytochemical and nutrient compositions as well as hypoglycemic properties of Acanthus montanus, Asystasia gangetica, Gongronema latifolium and Solanum melongenas have been reported in several previous studies. The present study sought to evaluate serum level of activity of the visceral organs toxicity marker enzyme (lactate dehydrogenase) as a measure of the capacities of single and combinatorial herbal formulations of A. montanus, A. gangetica, G. latifolium and S. melongenas to ameliorate systemic toxicity in alloxan-induced DM rats. Additionally, visceral organs and body weights were measured in order to ascertain the beneficial outcomes against organ inflammation or necrosis as well as general body tissue wasting in DM rats following administration of the herbal formulations.

MATERIALS AND METHODS

Collection and preparation of samples

Fresh leaves of A. montanus (Nees) T. Anderson (ACMO), A. gangetica L. T. Anderson (AGSA) and were collected from uncultivated lands in Umunwari-Iyinishi, Ikeduru Local Government Area, Imo State, Nigeria. Fresh leaves of G. latifolium Benth. (GOLA) and S. melongenas (SOME) were collected from a private garden in the above stated location. The leaves of the four plants were transported to the laboratory within 24 h, where they were identified and authenticated by Dr. E.N. Mbargwu at the Herbarium of the Department of Plant Science and Biotechnology, Imo
State University, Owerri, Nigeria. All the leaves were collected between the months of July and August, 2016.

Preparation of the leaves for extraction was according to the methods previously described. The leaves of individual plants were washed with continuous flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature (28 ± 2°C). A 500 g part of each herbal samples were weighed 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. The separate dried leaves were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA) and the ground leaves samples were stored in air-tight plastic bottles with screw caps pending extraction.

Preparation of leaf extracts

Preparation of ethanolic leaf extracts of the four ground samples was according to the methods previously described. A 40 g portion of each pulverized dried samples of ACMO, ASGA, SOME and ASGA were subjected to repeated soxhlet extraction cycles for 2 h using 96% C₂H₂OH (BDH, UK) as the solvent to obtain a final volume of 500 mL of each herbal extract. The separate volumes of the extracts were concentrated and recovered in a rotary evaporator (Büch Rotavapor R-200) 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: ACMO = 17.34% (w/w), ASGA = 18.12% (w/w), SOME = 19.39% (w/w), ASGA = 18.31% (w/w) and. The separate leaf extracts were reconstituted in phosphate buffered saline (PBS) solution, osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 Na₂HPO₄,2H₂O and 2.43 g NaH₂PO₄,2H₂O). Appropriate doses of the leaf extracts were prepared and administered to the rats.

Experimental animals/ethics

One hundred and two (102) male Wistar albino rats of weights within the range of 175.35 – 203.30 g were purchased from a commercial animal house in Owerri-North LGA, Imo State, Nigeria. The rats were housed in well-ventilated metal cages and maintained at room temperature (28 ± 2°C), 40–55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and pelletized standard Guinea Feed® (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria) ad libitum. They were kept for 2 weeks to acclimatize to environmental conditions. The present study was approved by the ethical committee on the use of animals for research, Department of Biochemistry, Federal University Technology, Owerri, Nigeria. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Induction of diabetes mellitus/experimental design

DM was induced in the rats by single intra-peritoneal (i.p) injection of 90 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) in PBS solution (pH = 7.4). The rats with fasting plasma glucose concentration (FPGG) > 5.71 mmol/L for 5 consecutive days were considered diabetic and selected for the study. A total of 102 male Wistar rats were divided 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. The animal groups were designated on the basis of treatments received by oral gavage on a daily basis for 21 days as previously described. Herbal extracts used for the treatments of the DM rats (DM-r) were designated as single herbal formulations (SHFs): (DM-rACMO, DM-rASGA, DM-rGOLA and DM-rSOME), double herbal formulations (DHF): (DM-rAGAM, DM-rAGGL, DM-rAGSM, DM-rAMGL, DM-rAMSM and DM-rGSLM), triple herbal formulations (THF): (DM-rAGGS, DM-rAGAM, DM-rAMAS and DM-rAMGS) and quadruple herbal formulation (QHF): (DM-rAGGS). The animal groups were designated on the basis of treatments received by oral gavage on a daily basis for 21 days as previously reported.

Serum lactate dehydrogenase activity

The heparinized non-haemolysed serum LDH activity of 12 h post-fasted rats was measured on the 21st day of treatment. Serum LDH activity was measured using Spectrum Diagnostics liquizyme LDH reagent/assay kit (MDSS GmbH Schiffgraben, Hannover, Germany) according to the manufacturer's instructions as previously reported.
Body weights

The body weights of the rats were measured using an electronic weighing balance (Digital Precision Weighing Balance (JCS-QC03) – China), on the day of commencement of treatment, and again on the 21st day. The body weights of the rats was calculated and reported in grams thus:

**Equation 1**

\[
\% \Delta b.w. = \frac{(b.w.\text{ \_AT}) - (b.w.\text{ \_BT})}{b.w.\text{ \_BT}} \times 100
\]

Where:  
\( \% \Delta b.w. \): Percentage change in body weight  
\( b.w.\text{ \_AT} \): Body weight after treatment on day 21  
\( b.w.\text{ \_BT} \): Body weight before treatment on day 0

**Visceral organs/body weight ratios**

Visceral organs (heart, pancreas, liver, spleen and right + left kidneys) weights were measured on day 21. The visceral organs were rinsed in 10% formolsaline, which was composed of 10 mL formalin (40 mL formaldehyde + 100 mL distilled water) in 90 mL PBS solution to remove blood constituents from the harvested visceral organs as previously described. The visceral organs were placed in between blotting papers and allowed to dry at 25 ± 2°C for 2 h before weighing. Organ weight was reported in grams per body weight. The visceral organs/body weight ratio was calculated thus:

**Equation 2**

\[
\text{Ratio o.w. : b.w.} = \frac{o.w.\text{ \_AT}}{b.w.\text{ \_AT}}
\]

Where:

\( o.w.:b.w. \): Organ weight to body weight ratio  
\( o.w.\text{ \_AT} \): Organ weight after treatment on day 21  
\( b.w.\text{ \_AT} \): Body weight after treatment on day 21

**Statistical and data analyses**

The data collected was analyzed by the analysis of variance procedure while treatment means shall be separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version, (2006).

**RESULTS**

Serum LDH activity of Group 2 was significantly higher (\( p < 0.05 \)) than that of Group 1 as well as herbal extract treated rat groups (Figure 1). Generally, serum LDH activities of the herbal extract treated rat groups varied within a relatively narrow range of 549.9 ± 12.10 – 500.6 ± 12.02 IU/L. For example, the serum LDH activity of Group 4, Group 5, Group 11, Group 13, Group 14, Group 15 and Group 16 showed no significant difference between these groups (\( p > 0.05 \)). Likewise, serum LDH activities of Group 3, Group 6 and Group 9 exhibited no significant difference (\( p > 0.05 \)) but were significantly different (\( p < 0.05 \)) from those of Group 4, Group 5, Group 11, Group 13, Group 14, Group 15 and Group 16. Serum LDH activities of the herbal extract treated rats were generally lower than that of Group 2, but were higher than that of Group 1; \( p < 0.05 \). Figure 2a shows that Group 1 exhibited significant increase (\( p < 0.05 \)) in body weight, whereas Group 2 a significant decrease (\( p < 0.05 \)) in body weight following treatment. The body weights of most experimental rat groups after herbal extract treatment were significantly higher (\( p < 0.05 \)) than their corresponding weights before herbal extract treatment, with the exception of the body weights of Group 3, Group 9, Group 15 and Group 17. These groups showed no significant difference (\( p > 0.05 \)). Group 6 exhibited significant decrease (\( p < 0.05 \)) in body weight, whereas that of Group 14 showed no significant decrease (\( p > 0.05 \)) in body weight following herbal extract treatment. An overview of percentage changes in body weights of the experimental rat groups is illustrated in Figure 2b. Group 2, Group 6, Group 13 and Group 14 exhibited negative percentage changes in body weights within the range of -6.01 ± 2.01% – (-1.18 ± 0.52%). Conversely, positive percentage changes in body weights of the experimental rat groups varied between 19.36 ± 4.21% and 1.06 ± 0.42%. Furthermore, the percentage change in body weight of Group 7 was 18.3 fold higher than that of Group 1; \( p < 0.05 \). The percentage change in body weight of Group 4 was not significantly different (\( p > 0.05 \)) from that of Group 5. Also, the percentage change in body weight of Group 10 was comparable with that of Group 1; \( p > 0.05 \). Group 3, Group 9, Group 11, Group 12, Group 15, Group 16 and Group 17 exhibited lower and marginal percentage changes in body weights when compared with Group 1.

Figure 3 shows that the ratio of heart weight to body weight of the experimental rat groups varied within a relatively narrow range: 0.0036 ± 1.1 x 10^-4 – 0.0048 ± 1.2 x 10^-4; \( p > 0.05 \). For example, the ratios of heart weights to body weights of Group 9 and Group 11 were 1.3 fold higher.
than that of Group 1. Furthermore, the ratios of heart weights to body weights of Group 6, Group 14 and Group 16 were equivalent to that of Group 2; p > 0.05.

The ratios of liver weights to body weights were within the range of 0.0293 ± 1.4 x 10⁻³ – 0.0597 ± 2.3 x 10⁻³ (Figure 3). Additionally, the ratio of liver weight to body weight of Group 2 was significantly higher (p < 0.05) than those of other experimental rat groups. However, the ratios of liver weights to body weights of herbal extract treated groups were significantly lower (p < 0.05) than that of Group 2. Conversely, the ratios of liver weights to body weights of herbal extract treated groups were comparable with that of Group 1 (p > 0.05), except for those of Group 5, Group 6 and Group 9; p < 0.05.

The ratio of kidney weight to body weight of Group 2 was 1.64 fold higher than that of Group 1; p > 0.05. Figure 3 shows that the ratios of kidney weights to body weights of herbal extract treated rat groups were significantly lower (p < 0.05) than that of Group 2, except those of Group 6 and Group 9; p > 0.05. The ratio of pancreas weight to body weight of Group 2 was 1.15 fold higher than that of Group 1 (p > 0.05). Additionally, the ratio of pancreas weight to body weight of Group 2 was comparable with those of herbal extract treated groups with exception to those of Group 3, Group 4, Group 5, Group 8, Group 10, Group 11 and Group 12, which gave comparatively lower numerical values.

DISCUSSION

Cells release LDH into systemic circulation after tissue damage or erythrocyte haemolysis. Accordingly, measurement of activities of marker enzymes (notably LDH activity) in serum is a reliable diagnostic parameter for ascertaining inherent-, biological- and xenobiotic-induced systemic toxicity. Elevated serum LDH activity is a reliable clinical indicator of organ injuries, especially those adversely affected by DM pathology and chemical toxicity, namely, the heart, liver and muscle. The relatively raised level of serum LDH activity was obvious indication of systemic toxicity occasioned by tissue necrosis as exemplified in alloxan-induced DM rat (Group 2; DIAB). In clinical diagnosis, LDH activity is relatively low in serum in absence of cellular injuries, whereas raised level of serum LDH activity is a reflection of tissue infarction and/or necrosis and parallels the number of necrotic cells. Previous reports have shown that RONS mediated tissue necrosis, as a result of alloxan/streptozotocin intoxication in concert with the pathophysiology of DM, contributed to organ injuries and was a reflection of raised levels of serum LDH activity in DM rats. In concord with previous findings, the present study showed that DHF, namely, combinatorial formulations of leaf extracts of A. gangetica + G. latifolium (200 mg/kg body weight), A. gangetica + A. montanus (200 mg/kg body weight) and A. montanus + G. latifolium (200 mg/kg body weight) exhibited relatively high capacities to reverse systemic toxicity engendered by alloxan-induced DM compared with other herbal formulations. Additionally, the outcome of the present study suggested that the presence of antioxidant phytochemicals in the individual leaf extract, A. gangetica, A. montanus and G. latifolium, effectively obliterated RONS, and thereby arrested tissue necrosis and ameliorated systemic toxicity in alloxan-induced DM rats.

Empirical investigations revealed that DM is associated with weight loss in human and animal models. One striking pathologic feature of DM is impeded carbohydrate metabolism, which engenders distortion in general metabolism such that the alternative energy needs of the body system is derived from intense catabolism of structural protein molecules of the muscle as well as neutral lipid stored away in adipose tissues. These altered metabolic events result in rapid loss of body weight due to the increased muscle and adipose tissues wasting, as typified in alloxan-induced DM rat (Group 2; DIAB). The increases in body weights of the DM rats treated with single and combinatorial herbal formulations, as opposed to the untreated DM rats, were indications of the capacity of the leaf extracts to ameliorate DM pathology and were in conformity with previous reports. Changes in visceral organs weights as well as other anatomical aberrations have provided useful information in predicting and establishing pathologic conditions in animal models. The use of organ weight as a diagnostic parameter is predicated on the fact that pathologic conditions that affect body weight will also affect visceral organs weights of the animal. Previous studies have shown that streptozotocin intoxication increased kidney and liver weights, with corresponding decreases in body weights of the DM rats, whereas the pancreas weight was not affected. The findings of the present study conformed to the report of Zafar and Naqvi, which showed that the untreated alloxan-induced DM rats (Group 2; DIAB) exhibited increased in liver and kidney weights (hypertrophy), whereas there were no profound changes in the weights of the pancreas and heart. According to Malatiali et al., diabetic glomerular hypertrophy constitutes an early event in the progression of glomerular pathology. Although the mechanism of renal hypertrophy has not been established, Sharma and Ziyadeh, proposed that development of renal hypertrophy in Type I DM was linked to overexpression of transforming growth factor (TGF) in the renal tissues, especially in proximal convoluted tubules cells and glomerular mesangial cells. Another report attributed renal hypertrophy and hyperplasia to increased...
epidermal growth factor (EGF). Several other proposed mechanisms leading to the development and progression of renal hyperplasia have been exhaustively reported elsewhere. The present study showed that single and combinatorial herbal formulation reversed renal tissue hypertrophy which ultimately could protect the renal tissues against nephropathy. Previous studies also show the capacity of methanolic leaf extract of Punica granatum to reverse diabetic nephropathy. Liver hypertrophy may be due to increased mobilization of free fatty acids to liver, which engender fatty liver as well as a low capacity of the hepatocytes to secret lipoprotein as a result of compromised capacity of the hepatocytes to biosynthesize apoprotein B in DM state. The present study showed that single and combinatorial herbal formulations protected the liver against liver hypertrophy in alloxan-induced DM rats. However, administration of SHFs, namely, G. latifolium (200 mg/kg body) and S. melongena (200 mg/kg body weight) as well as DHF- A. gangetica + S. melongena (200 mg/kg body weight) caused marginal reduction in liver hypertrophy in alloxan-induced DM rats.

CONCLUSION

The present study showed that the specified dose of 200 mg/kg body weight of single and combinatorial herbal formulations administered to alloxan-induced DM rats lowered serum LDH activity, which was an indication of protective effect of the herbal formulations against RONS-induced visceral organs necrosis as well as their capacities to ameliorate systemic toxicity. In addition, the levels of reversal of loss in body weight, as well as the regression of liver and renal tissues hypertrophy in alloxan-induced DM rats following the administration of single and combinatorial herbal formulations depended on the type and number of individual herbal extract used in the herbal formulations. Overall, 200 mg/kg body weight DHFs of A. gangetica + A. montanus and A. gangetica + G. latifolium offered the greatest therapeutic benefits, with respect to all diagnostic parameters considered in the present study, to alloxan-induced DM rats.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ABBREVIATIONS USED

NaCl: Sodium chloride; NaHPO₄·2H₂O: Disodium hydrogen phosphate dihydrate; NaH₂PO₄·2H₂O: Sodium dihydrogen phosphate dihydrate.

REFERENCES

33. Murugan P, Pari L. Effect of tetrahydrocurcumin on plasma antioxidants in strep-
Chikezie, et al.: Serum lactate dehydrogenase activity and visceral organs and body weights of diabetic rats

SUMMARY

A dose of 200 mg/kg body weight of single and combinatorial herbal formulations administered to alloxan-induced DM rats exhibited protective effect against RONS-induced visceral organs necrosis as well as ameliorated systemic toxicity.

The levels of reversal of loss in body weight, as well as the regression of liver and renal tissues hypertrophy in alloxan-induced DM rats following the administration of single and combinatorial herbal formulations depended on the type and number of individual herbal extract used in the herbal formulations.

A dose of 200 mg/kg body weight DHFs of A. gangetica + A. montanus and A. gangetica + G. latifolium offered the greatest therapeutic benefits to alloxan-induced DM rats.

PICTORIAL ABSTRACT

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