ORIGINAL ARTICLE

Histopathological studies of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations

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Background and Aim: The present study investigated the capacity of single and combinatorial herbal formulations of leaf extracts of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, and *Hibiscus rosasinensis* to reverse renal and hepatic injuries in alloxan-induced hyperglycemic rats. **Settings and Design:** A total of 102 male Wistar rats were allotted into 17 groups of six rats each. The animals were deprived of food and water for additional 16 h before the commencement of treatment. The animal groups were designated on the basis of treatments received at regular intervals of 2 days for 30 days. **Materials and Methods:** Histological images of renal and hepatic tissue sections were captured using charge-couple device camera under a light microscope. Blood biochemical indices were measured using spectrophotometric methods. **Statistical Analysis:** The data were analyzed by one-way ANOVA followed by Dunnett test, with the level of significance set at $P < 0.05$. **Results:** Histopathological studies of the untreated hyperglycemic rats showed evidence of hypertrophy and disarrangements of the hepatic parenchyma. The architecture of hepatic parenchyma showed evidence of varying levels of necrosis and restoration of cellular integrity following herbal treatment. Similarly, histopathological studies of the renal tissues showed evidence of cells swellings and fibrotic changes of the glomeruli. However, herbal treatments restored cellular integrity and reversed glomeruli atrophy and turf disarrangement. In addition, blood biochemical indices showed evidence of restoration of cellular integrity. **Conclusion:** Both histological and biochemical indices revealed varying capacities of single and combinatorial herbal formulations to reverse renal and hepatic tissues injuries in hyperglycemic rats.

Key words: Hepatic, herbal formulations, histology, hyperglycemia, renal

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder associated with poor secretion of insulin or resistance to insulin actions by peripheral tissue.[1] Studies have established a connection between type 1 DM and compromised activities of reactive oxygen species antagonists and scavenging enzymes,[2] which engender disturbances in metabolism[3] with attendant oxidative stress induced tissue damage and complications such as retinopathy, microangiopathy, ketoacidosis, neuropathy, and nephropathy. Molecular events leading to β-cell dysfunction and insulin resistance are connected with stress-sensitive signaling pathways, which are progenitors of DM pathology and complications.[4] Since alloxan or streptozotocin causes selective oxidative damage to pancreatic β-cells, intra-peritoneal (i.p) injection of the salt solution is commonly used to induce type 1 DM in experimental animals.

Acanthus montanus is traditionally used in Nigeria for the management of DM and several diseases. *Asystasia gangetica* is used in ethnomedicinal practices for the alleviation of asthma, platelet aggregation, inflammation, and rheumatism.[5] The medicinal usefulness of *Emilia coccinea* has been reviewed in the reports of Edeoga *et al*.[6] The ethnomedicinal usefulness of herbal extracts of *Hibiscus rosasinensis* has been exhaustively reported by Kumar *et al*.[7] Histopathological studies are useful tools for identification and characterization of organ injury and recovery. In addition, biochemical

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Received: 28-01-2015; **Accepted:** 06-05-2015

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.161237

How to cite this article: Ojiako OA, Chikezie PC, Ogbuji AC. Histopathological studies of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations. Int J Green Pharm 2015;9:184-91.

tests such as kidney function test and liver function test are diagnostic parameters for ascertaining organ functionality and level of recovery from injuries during the course of therapeutic intervention.

The present study investigated the capacity of single and combinatorial herbal formulations of leaf extracts of *A. montanus*, *A. gangetica*, *E. coccinea*, and *H. rosasinensis* to reverse renal and hepatic injuries in alloxan-induced hyperglycemic rats.

MATERIALS AND METHODS

Collection and Preparation of Herbal Samples

Fresh leaves of *A. montanus* (Nees) T. Anderson (ACMO726), *E. coccinea* G. Don (EMCO637), and *H. rosasinensis* L. (HIRO392) were collected from uncultivated lands in Umuamacha Ayaba Umaeze, Osisioma Ngwa Local Government Area (LGA), Abia State, Nigeria, whereas fresh leaves of *A. gangetica* L. T. Anderson (ASGA477) were collected from Ubowuala, Emekuku, Owerri North LGA, Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe, School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri. Voucher specimens were deposited at the Herbarium for reference purposes. All the leaves were collected between the months of July and August, 2009.

The leaves of individual plants were washed with a continuous flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature (24°C ± 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 a cold room (7°C ± 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, UK) as solvent to obtain a final volume of 500 mL of each herbal extracts. These volumes of the herbal 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), *A. gangetica* = 16.69% (w/w), *E. coccinea* = 17.99% (w/w), and *H. rosasinensis* = 17.23% (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.

Experimental Animals

Male albino (Wistar) rats weighing between 150 and 160 g were maintained at room temperatures of 24°C ± 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 (1978).

Induction of Diabetes/Experimental Design

Hyperglycemia was induced in the experimental rats by single i.p injection of 90 mg/kg body weight of alloxan monohydrate (Sigma, St. Louis, USA) in PBS solution (pH = 7.4). The animals with fasting plasma glucose concentration >110 mg/dL after 72 h were considered hyperglycemic and selected for the study. A total of 102 male Wistar rats were allotted into 17 groups of six rats each. The animals were deprived of food and water for additional 16 h before the commencement of treatment. The animal groups were designated on the basis of treatments received at regular intervals of 2 days for 30 days. Herbal treatments of the hyperglycemic rats were described as single herbal formulations (SHfs): (Group 3, Group 4, Group 5, and Group 6), double herbal formulations (DHfs): (Group 7, Group 8, Group 9, Group 10, Group 11, and Group 12), triple herbal formulations (THfs): (Group 13, Group 14, Group 15, and Group 16) and quadruple herbal formulation (QHf): (Group 17).

- Group 1: Normal rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS
- Group 2: Hyperglycemic rats received SCF + water *ad libitum* + 1.0 mL/kg of PBS
- Group 3: Hyperglycemic rats received SCF + water *ad libitum* + *A. montanus* (20 mg/kg in PBS; i.p.)
- Group 4: Hyperglycemic rats received SCF + water *ad libitum* + *A. gangetica* (20 mg/kg in PBS; i.p.)
- Group 5: Hyperglycemic rats received SCF + water *ad libitum* + *E. coccinea* (20 mg/kg in PBS; i.p.)
- Group 6: Hyperglycemic rats received SCF + water *ad libitum* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- Group 7: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *A. gangetica* + *A. montanus* (20 mg/kg in PBS; i.p.)
- Group 8: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *A.*

gangetica + *E. coccinea* (20 mg/kg in PBS; i.p.)
- Group 9: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *A. gangetica* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- Group 10: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *A. montanus* + *E. coccinea* (20 mg/kg in PBS; i.p.)
- Group 11: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *A. montanus* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- Group 12: Hyperglycemic rats received SCF + water *ad libitum* + combined dose (ratio: 1:1 w/w) of *E. coccinea* + *H. rosasinensis* (20 mg/kg in PBS; i.p.)
- Group 13: Hyperglycemic rats 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.)
- Group 14: Hyperglycemic rats 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.)
- Group 15: Hyperglycemic rats 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.)
- Group 16: Hyperglycemic rats 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.)
- Group 17: Hyperglycemic rats 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.).

Histopathological Examinations

Organ histology was according to the methods described by Banchroft *et al.*[8] Photomicrographs of the tissue sections were captured using charge-couple device camera under a light microscope (Olympus BX51TF; Olympus Corporation, Tokyo, Japan) at ×400 magnification power.

Determination of Biochemical Parameters

Creatinine

Measurement of serum creatinine concentration was according to the methods as described by Bonsnes and Taussky.[9]

Urea

Estimation of serum urea concentration was by the rapid method as described by Fawcett and Scott.[10]

Aspartate Aminotransferase and Alanine Aminotransferase Activities

Measurement of serum Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were according to the methods of Reitman and Frankel.[11]

Alkaline Phosphatase Activity

Serum alkaline phosphatase (ALP) activity was assayed by the methods described by Njoku *et al.*[12]

Bilirubin

Serum total bilirubin concentration was measured using diazotized sulfanilic acid methods as previously described.[13]

Statistical Analysis

The results were expressed as mean ± standard error of the mean, and statistically analyzed by one-way ANOVA followed by Dunnett test, with the level of significance set at $P < 0.05$.

RESULTS

Histopathological studies showed Group 1 exhibited normal cellular integrity and normal lobular architecture with central veins and radiating cords of hepatocytes, separated by blood sinusoids, which was comparable to that of Group 8 and Group 17 [Figure 1]. Conversely, Figure 1 also showed evidence of hypertrophy and disarrangements of hepatic parenchyma in Group 2, Group 4, Group 9, Group 11, and Group 10 with obvious histological changes, typified by distortions in hepatic organization. The hepatic tissues of some treated hyperglycemic rats groups (Group 4, Group 10, and Group 13) displayed minor necrosis and fibrotic changes. Similarly, Group 2 and Group 14 showed conspicuous evidence of fatty deposits. Similarly, histopathological studies of the renal tissues of Group 1 showed comparable physiologic features with that of Group 4, Group 9, and Group 11. Renal tissues of Group 2 and hyperglycemic rats treated with herbal formulations (Group 3, Group 5, Group 6, Group 7, Group 10, Group 12, Group 13, Group 14, Group 15, and Group 16) showed evidence of cells swellings and congestion, which was an indication of thickening of renal vesicles and fibrotic changes of the glomeruli [Figure 2]. However, these were indications of the capability the herbal formulations to restore cellular integrity and reverse glomeruli atrophy and turf disarrangement as exemplified by the histological features of Group 4, Group 8, Group 9, Group 11, and Group 17.

Serum creatinine concentration of Group 2 was significantly ($P < 0.05$) higher than that of Group 1 [Figure 3]. Serum creatinine concentrations of hyperglycemic rats treated with THfs were not significantly ($P > 0.05$) different from that of Group 1.

Figure 4 showed that the serum urea concentration of Group 2 was significantly ($P < 0.05$) elevated than that of Group 1. The serum urea concentrations of hyperglycemic

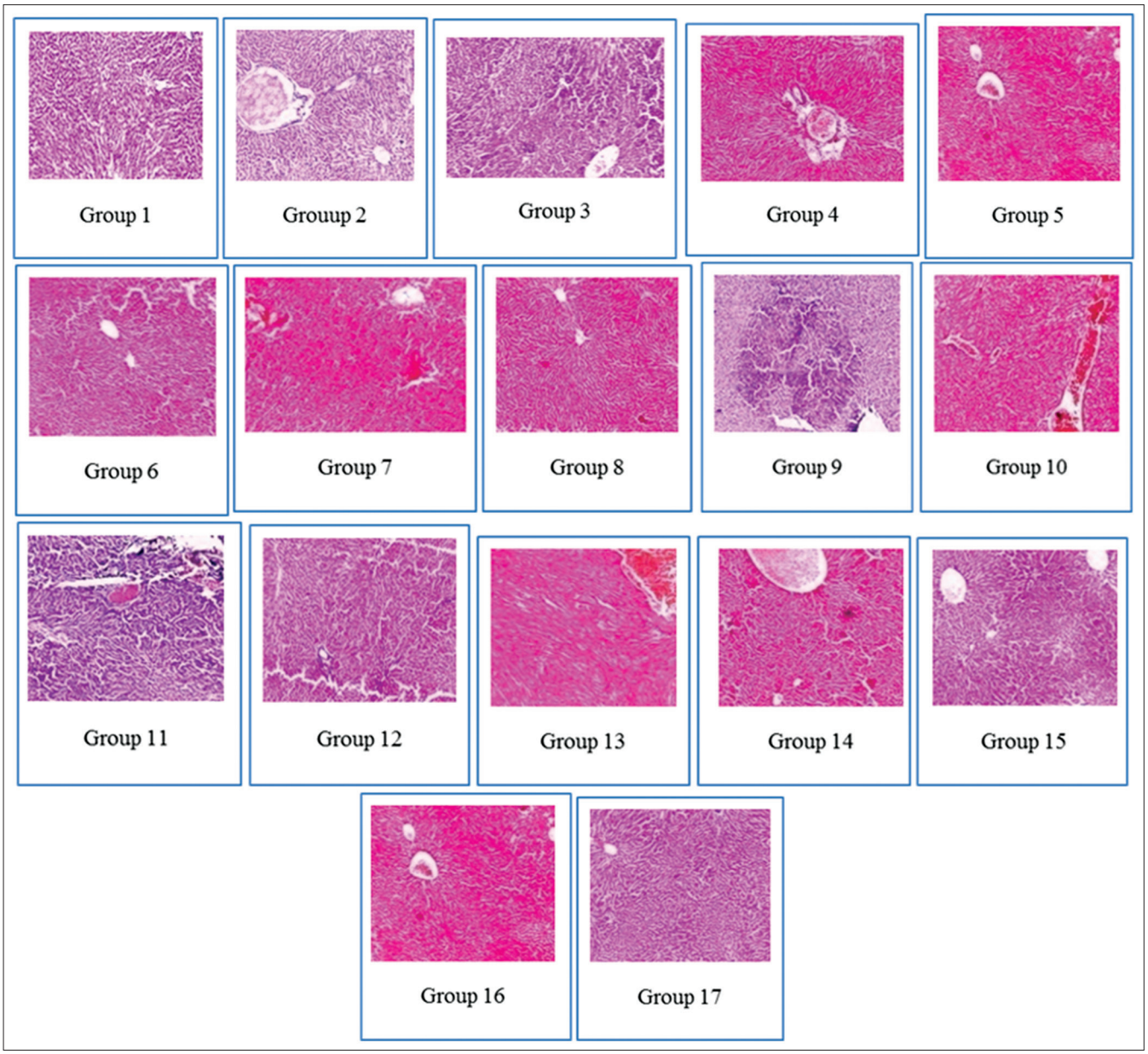

Figure 1: Histopathological images of liver sections of Group 1, Group 2, and hyperglycemic rats treated with single herbal formulations: (Group 3, Group 4, Group 5, and Group 6), double herbal formulations: (Group 7, Group 8, Group 9, Group 10, Group 11, and Group 12), triple herbal formulations: (Group 13, Group 14, Group 15, and Group 16), and quadruple herbal formulation: (Group 17) for 30 days. The architecture of hepatic parenchyma showed evidence of varying levels of necrosis and restoration of cellular integrity. Group 1, Group 8, and Group 17 showed normal histology

rats treated with DHfs did not show significant ($P > 0.05$) variability. Comparative analyses showed that serum urea concentrations of Group 7 and Group 8 were among the lowest values of serum urea concentrations, which corresponded to 3.28 and 3.83 folds lower than that of Group 2, respectively. Serum urea concentrations of Group 14, Group 15, and Group 16 were lower than that of Group 1.

Serum AST, ALT, and ALP activities of Group 2 were significantly ($P < 0.05$) higher than that of corresponding Group 1 [Figure 5]. Serum AST of hyperglycemic rats treated with SHfs showed lower levels of enzyme activities than that of Group 1. Serum ALT activity of Group 2 was significantly ($P < 0.05$) higher than that of Group 1 [Figure 5]. Furthermore, Figure 5 showed that serum ALP activity of Group 2 was significantly ($P < 0.05$) elevated than that of Group 1. Furthermore, Serum total bilirubin concentration of hyperglycemic rats treated with SHfs was within the narrow range of 12.5 ± 1.3 mol/L–13.5 ± 0.9 mol/L and comparable with serum total bilirubin concentration of Group 2. Serum total bilirubin concentration of Group 9, Group 17, and Group 11 were equivalent to that of Group 2. Finally, serum total bilirubin concentration of hyperglycemic rats treated with THfs was lower than that of Group 2 as $P > 0.05$.

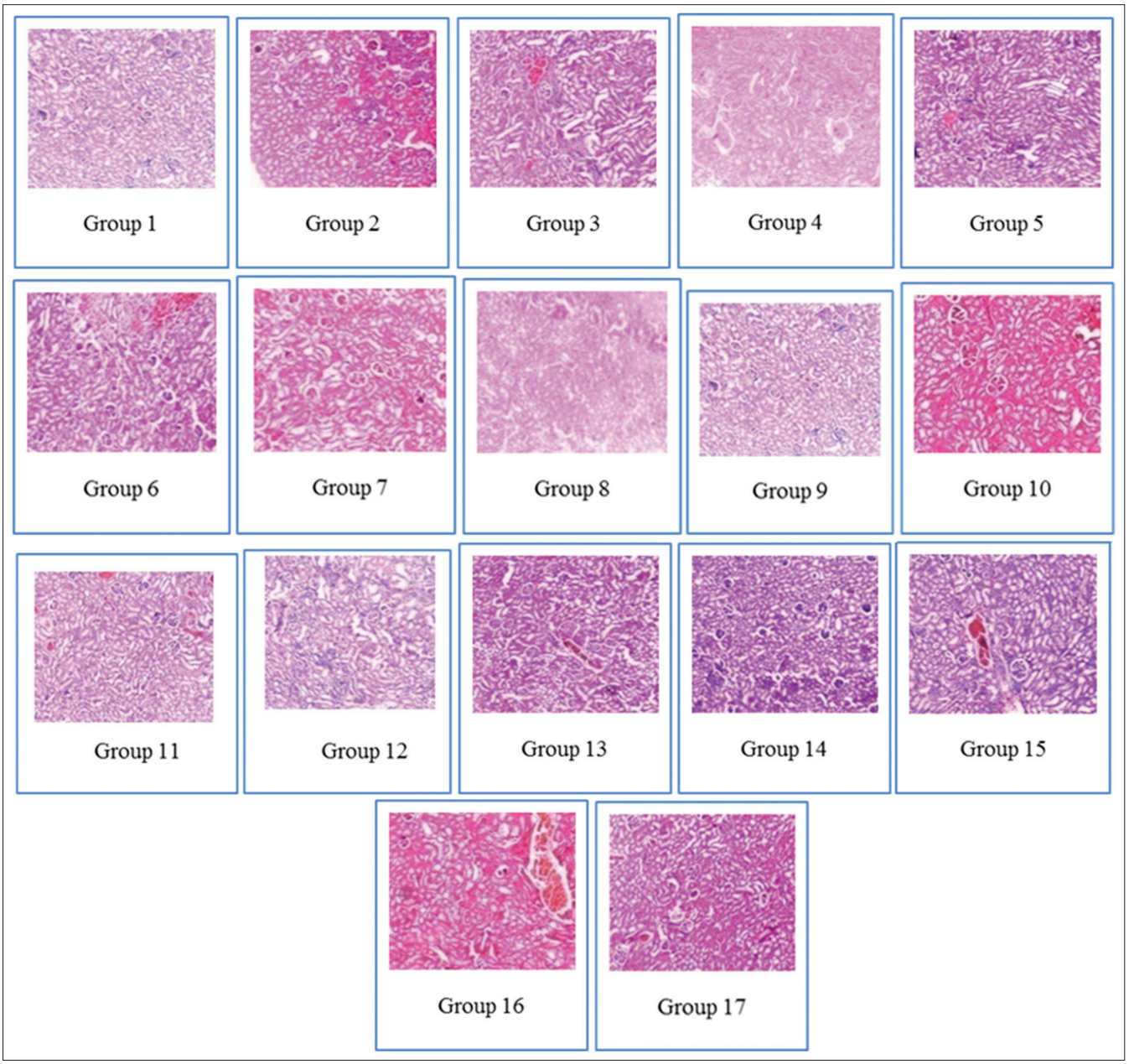

Figure 2: Histopathological images of renal tissue sections of Group 1, Group 2, and hyperglycemic treated with single herbal formulations: (Group 3, Group 4, Group 5, and Group 6), double herbal formulations: (Group 7, Group 8, Group 9, Group 10, Group 11, and Group 12), triple herbal formulations: (Group 13, Group 14, Group 15, and Group 16), and quadruple herbal formulation: (Group 17) for 30 days. Renal histology demonstrated tubular, corpuscular, and interstitial alterations. The architecture of renal parenchyma showed evidence of varying levels of necrosis and subsequent restoration of cellular integrity. Group 1 and Group 9 showed normal histology

DISCUSSION

The present study confirmed the reliability of histopathological methods and biochemical indices in ascertaining organ integrity and functionality as previously described. More so, the underlying molecular mechanisms that engender distortions of renal and hepatic tissues morphology, with attendant tissue damage, in pathological and chemical induced diabetic animal models have been widely and exhaustively reported and corroborated the observations of the present investigations. Biological and chemical induced alterations in tissue architecture elicit metabolic disorders with attendant pathology, which could be ameliorated or reversed by herbal remedies.[14] For instance, according to Koneri *et al.*,[15] saponin isolated from *Momordica cymbalaria* Fenzl caused considerable quantitative increase and rejuvenation of β-cells (75%) of streptozotocin-induced diabetic rats, in which oleanane-type triterpenoid saponin was implicated as the active anti-diabetic principle of the plant extract. Another study by El-Soud *et al.*,[16] also reported the capability of alkaloid extract of fenugreek dried seeds (*Trigonella foenum-graecum* L.) to exert glycemic control and reverse renal and hepatic tissues damage in streptozotocin induced

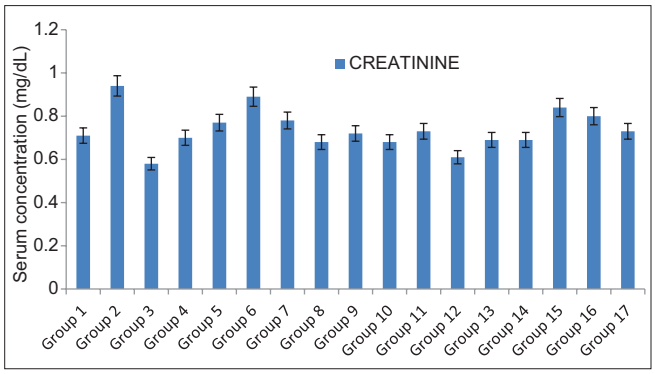

Figure 3: Serum creatinine concentrations of normal, diabetic, and treated rats

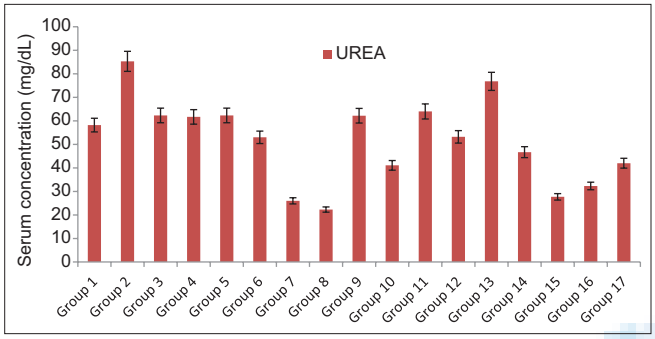

Figure 4: Serum urea concentrations of normal, diabetic, and treated rats

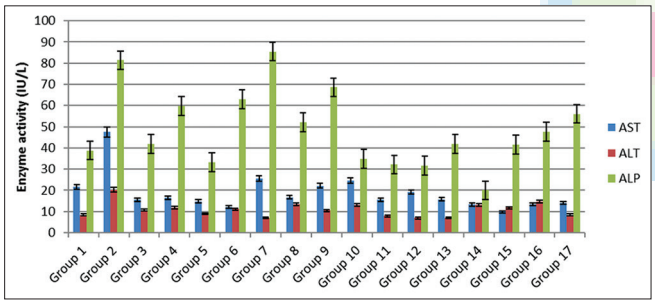

Figure 5: Serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities of normal, diabetic, and treated rats

hyperglycemic rats. Accordingly, the present study showed that some of the experimental herbal formulations displayed noticeable capacities to reverse renal and hepatic tissues degeneration and disarrangement, typified by Group 4, Group 9, and Group 11 [Figure 1], Group 8 and Group 17 [Figure 2].

There are established evidence in support of the fact that structural distortions and damage to tissues of diabetic animals are the outcome of excessive production of free radicals and oxidative stress.[17] The majority of plant bioactive principles are free radical and oxidative stress antagonist and therefore, herbal products are potent therapeutic agents for the alleviation and prevention of pathologic conditions whose etiology is potentially or directly linked to oxidative stress. In that regard, the varying capabilities of the various single and polyherbal formulations to reverse renal and hepatic tissue damages [Figures 1 and 2] are not unconnected with the outcomes of phytochemical interactions among the composite herbal extracts, which may either display synergy or antagonism in terms of their therapeutic actions as previously described.[18]

Elevations of blood creatinine and urea concentrations are diagnostic of renal dysfunction and creatinine concentration parallels that of urea.[19] Therefore, pathologic conditions that impair renal function cause raised blood creatinine and urea levels. It is worthwhile to note that raised blood creatinine and urea levels are the outcome of longstanding DM complications leading to diabetic nephropathy.[20] However, using clinical survey protocol, Hjelmesæth *et al.*,[21] proposed a relationship between low serum creatinine levels and type 2 DM, in which they noted that low skeletal muscle mass was inversely associated with insulin resistance and metabolic syndrome. This proposed relationship was consistent with previous report,[22] which noted that the absolute glomerular filtration rate was higher in severely obese subjects than in their corresponding lean counterparts, and therefore, associated with lower serum creatinine levels. Histological and biochemical investigations of the present study revealed evidence of alterations of renal tissues morphology with compromised functionality. However, renal dysfunction in the experimental rats was reversed following therapeutic intervention with some of the herbal formulations, as exemplified in treated hyperglycemic rats of the following categories: Group 4, Group 9, Group 11, Group 8, and Group 17. Related studies have also established the capability of an herbal extract of *Vernonia amygdalina* to rejuvenate and protect renal tissues of alloxan-induced diabetic mice.

Nannipieri *et al.*,[23] had proposed the use of level of serum activity of enzymes conventionally associated with hepatic dysfunction, occasioned by tissue injuries, as indices for diagnosis and prediction of incidence of DM. According to Nannipieri *et al.*,[23] mild elevations of hepatic enzymes occurred in plasma of DM patients; of which only raised γ-glutamyl transferase activity was an independent predictor of impaired glucose tolerance, especially in type 2 DM. Furthermore, West *et al.*,[24] reported the incidence of raised levels of serum ALT activity in type 1 and type 2 DM. In addition, the use of ALP isoform band 10 (ALP-10) in the screening of autoimmune disorders, as in the in case of type 1 DM, has been described elsewhere.[25] They further noted that serum of patients with early type 1 DM and degenerative disorders such as rheumatoid arthritis and multiple sclerosis exhibited significant increases in serum levels of ALP and particularly, ALP-10 activities.

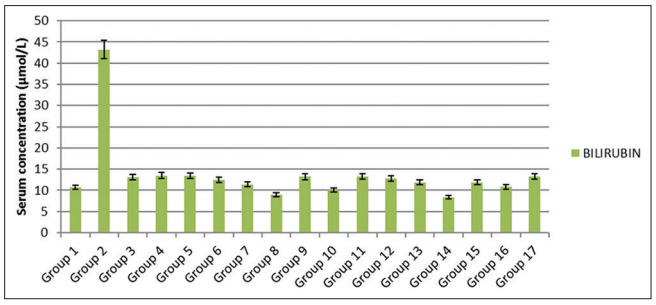

Figure 6: Serum total bilirubin concentrations of normal, diabetic, and treated rats

Raised level of serum ALP activity supports the metabolic etiology of bone disease in DM.[26] Although the absolute level of activity of serum AST > ALT,[27] serum ALT activity is more specific for hepatic injury than an increase in serum AST level. Furthermore, raised serum level ALT activity may reflect fatty changes in the liver as in the case of nonalcoholic fatty liver disease as corroborated by the present findings [Figure 1], which was a classical type 1 DM prototype, and in type 2 DM.[28] The results of the present study showed evidence of raised levels of hepatic enzymes (AST, ALT, and ALP) activities in serum of untreated hyperglycemic rats when compared with those treated with the various herbal formulations. Therefore, the experimental herbal extracts displayed varied capacities to ameliorate hepatic injury in hyperglycemic rats.

The relatively higher serum bilirubin concentration of untreated hyperglycemic rats [Figure 6] may have arisen from failure to conjugate bilirubin following a compromised functional integrity of the hepatocytes as a result of DM induced injury. Nevertheless, studies have shown that bilirubin is an endogenous antioxidant with protective actions against oxidative stress-induced renal tissues injuries;[29] rejuvenates of endothelial function in type 2 DM.[30] Specifically, reports showed that higher serum bilirubin levels, within the normal range, might be predictive of lower risk of progression toward nephropathy in type 2 DM. It thus appears that serum bilirubin levels fulfill dual criteria for clinical diagnosis and physiologic therapeutic intervention strategy.

CONCLUSION

From the results of the present study, both histological and biochemical indices revealed varying capacities of single and combinatorial herbal formulations to reverse and protect renal and hepatic tissues against DM induced organ injuries. Specifically, (DHf)-*A. gangetica* + *E. coccinea* and (QHf)-*A. montanus* + *A. gangetica* + *E. coccinea* + *H. rosasinensis* exhibited hepatic protective effects, whereas (SHf)-*A. gangetica*, (DHf)-*A. gangetica* + *H. rosasinensis* and (DHf)-*A. montanus* + *H. rosasinensis* ameliorated renal tissue injuries in hyperglycemic rats.

Acknowledgments

The authors are thankful to Dr. M. Ibe, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

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