Research Article

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Blood indices/histopathological status of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations

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Abstract

Background: 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 (HyGR).

Materials and Methods: Hyperglycemia was induced in the experimental rats by single intra-peritoneal (i.p) injection of 90 mg/kg bw of alloxan monohydrate in phosphate saline buffered solution (pH = 7.4). Histological images of renal and hepatic tissue sections were captured using chare-ouple device (CCD) camera under light microscope. Blood biochemical indices were measured using spectrophotometric methods. Results: Histopathological studies of the untreated HyGR showed evidence of hypertrophy and disarrangements of hepatic parenchyma with obvious distortions in hepatic organization. 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 congestion, which was an indication of thickening of renal vessels and fibrotic changes of the glomeruli. However, there were indications of the capability the herbal formulations to restore cellular integrity and reverse glomeruli atrophy and turf disarrangement. Additionally, blood biochemical indices showed evidence of varied levels of restoration of cellular integrity and combinatorial herbal formulations to reverse renal and hepatic tissues injuries in HyGR.

Keywords: 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 tissues.1-3 The multifaceted etiology of DM has been described elsewhere.4-9 Studies have established a connection between Type 1 DM and compromised activities of reactive oxygen species (ROS) antagonists and scavenging enzymes10,11, which engender disturbances in metabolism12,13 with attendant oxidative stress induced tissue damage14 and complications such as retinopathy, microangiopathy, ketoacidosis, neuropathy and nephropathy.11,15-18 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.2,12,19 Since alloxan or streptozotocin causes selective oxidative damage to pancreatic β-cells, intra-peritoneal injection of their salt solutions is commonly used to induce Type 1 DM in experimental animals.3,17,20,21

The hepatocyte performs more than 500 metabolic transformations and serves as a storage site for glycogen, hydrophobic and hydrophilic vitamins.22 The dominant role of the hepatocyte in transformation and elimination of noxious chemicals exposes the liver to toxic injury.23 The kidneys regulate blood ions and pH levels as well as water balance. In addition, the kidneys serve as principal organ for the elimination of metabolic waste products. The functional unit of the kidney is the nephron and each kidney contains approximately two million of these structures. Histopathological studies are useful tools for identification and characterization of organ injury and recovery. Additionally, biochemical tests such as kidney function test (KFT) and liver function test (LFT) are diagnostic parameters for ascertaining organ functionality and level of recovery from injuries during the course of therapeutic intervention.
Acanthus montanus is traditionally used in Nigeria for the management of DM and several diseases. Asystasia gangetica is an antimicrobial used in ethnomedicinal practices for the alleviation of asthma, rheumatism, inflammation, platelet aggregation and DM. The medicinal usefulness of Emilia coccinea has been reviewed in the reports of Edeoga et al. Herbal extracts of Hibiscus rosasinensis are hypotensive agents and its ethnomedicinal usefulness has been exhaustively reported by Kumar et al. The phytochemical contents of the four aforementioned herbal extracts have been previously reported.

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 (HyGR).

Materials and Methods

Collection and preparation of herbal samples

Fresh leaves of Acanthus montanus (Nees) T. Anderson (ACMO), Emilia coccinea G. Don (EMCO) and Hibiscus rosasinensis L. (HIRO) were collected from uncultivated lands in Umunama Ayaba Umaeze, Osisioma Ngwa LGA, Abia State, Nigeria, whereas fresh leaves of Asystasia gangetica L. T. Anderson (ASGA) were collected from Ubowaala, Emekuku, Owerri North LGA, Imo State, Nigeria. The four herbs were identified and authenticated by Dr. M. Ibe, 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 continues 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 (WTC BINDER, 7200 Tuttlingen, Germany) at 60 °C. The leaves were refrigerated in airtight plastic bottles with screw caps pending extraction.

Extraction of herbal samples

Portion of 40 g of each pulverized dried samples of A. montanus, A. gangetica, E. coccinea and H. rosasinensis were subjected to repeated Soxhlet extraction cycles for 2 h using 96% C2H5OH (BDH, U.K) as solvent to obtain 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 airtight 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), H. rosasinensis = 17.23% (w/w) and. The separate extracts were reconstituted in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 Na2HPO4·2H2O and 2.43 g NaH2PO4·2H2O), before appropriating doses were administered to the experimental animals.

Experimental animals

Male albino (Wistar) rats weighing between 150-260 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 week 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

Hyperglycemia was induced in the experimental 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 (FGPC) > 110 mg/dl after 72 h were considered hyperglycemic 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. The animal groups were designated on the bases of treatments received at regular intervals of 2 days for 30 days. Herbal treatments of the HyGR were described as single herbal formulations (SHF): (HrACMO, HrASGA, HrEMCO and HrHIRO), double herbal formulations (DFH): (HrAGAM, HrAGEC, HrAMHC and HrECHR), triple herbal formulations (THF): (HrAEGH, HrAMAE, HrAMAH and HrAMEH) and quadruple herbal formulation (QHF): (HrAAEH).

- NORM: Normal rats receiving SCF + water ad libitum + 1.0 mL/kg of PBS.
- DIAB: HyGR receiving SCF + water ad libitum + 1.0 mL/kg of PBS.
- HrACMO: HyGR receiving SCF + water ad libitum + A. montanus (20 mg/kg in PBS; i.p.).
- HrASGA: HyGR receiving SCF + water ad libitum + A. gangetica (20 mg/kg in PBS; i.p.).
- HrEMCO: HyGR receiving SCF + water ad libitum + E. coccinea (20 mg/kg in PBS; i.p.).
- HrHIRO: HyGR receiving SCF + water ad libitum + H. rosasinensis (20 mg/kg in PBS; i.p.)
- HrAGAM: HyGR receiving 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 receiving SCF + water ad libitum + combined dose (ratio: 1:1 w/w) of A. gangetica + E. coccinea (20 mg/kg in PBS; i.p.).
- HrAEGH: HyGR receiving 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. gangetica + 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. gangetica + E. coccinea + H. rosasinensis (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 + E. coccinea + 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 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 Bancroft et al. Autopsy samples were taken from the renal and hepatic tissues of the different animal groups, fixed in 10% formol saline (pH = 7.2) for 24 h and washed with continuous flow of distilled water. The specimens were cleared in xylene embedded in paraffin in hot air oven at 56 °C for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4-mm thickness using a semi-automated rotatory microtome. The obtained tissue sections were collected on glass slides, dehydrated by immersing in serial dilutions of ethyl alcohol-water mixture, cleaned in xylene and embedded in paraffin wax. Next, the specimens were deparaffinized and stained with hematoxylin and eosin (H & E) dye for histopathological examinations. Photomicrographs of the tissue sections were captured using share-couple device (CCD) camera under light microscope (Olympus BX51TF; Olympus Corporation, Tokyo, Japan) at × 400 magnification power.

Creatinine

Measurement of serum creatinine concentration (SCC) was according to the methods as described by Bonsnes and Taussey. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities

Measurement of serum AST and ALT activities were according to the methods of Reitman and Frankel. Alkaline phosphatase (ALP) activity

Serum ALP activity was assayed by the methods described by Glogowski et al., but with minor modifications according to Njoku et al.

Bilirubin

Serum total bilirubin concentration (STBC) was measured using diazotized sulphanilic acid methods as previously described.

Statistical analysis

The results were expressed as mean ± SEM, and statistically analyzed by one way ANOVA followed by Dunnett test, with level of significance set at p < 0.05.

Results

Histopathological studies showed the NORM group 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 HrAGEC, HrAMHR and HrAAEH groups (Figure 1). Conversely, Figure 1 also showed evidence of hypertrophy and disarrangements of hepatic parenchyma of the DIAB, HrASGA, HrAGHR, HrAMHR and HrAAEC groups with obvious histological changes, typified by distortions in hepatic organization. The hepatic tissues of some treated HyGR groups (HrASGA, HrAMEC and HrAGEC and HrAGEH) displayed minor necrosis and fibrotic changes. Similarly, DIAB and HrAMAE showed conspicuous evidence of fatty deposits.

Similarly, histopathological studies of the renal tissues of the NORM group showed comparable physiologic features with that of the HrASGA, HrAGHR, HrAMHR groups. Renal tissues of DIAB group and HyGR treated with herbal formulations (HrACMO, HrEMCO, HrHIRO, HrAGAM, HrAMEC, HrECHR, HrAGEH, HrAMAE, HrAMAH and HrAMEH) 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 the HrASGA, HrAGEC, HrAGHR, HrAMHR and HrAAEH groups.
Figure 1: Histopathological images of liver sections of normal rats (NORM), untreated HyGR (DIAB) and HyGR treated with 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) for 30 days. The architecture of hepatic parenchyma showed evidence of varying levels of necrosis and restoration of cellular integrity. The NORM, HrAGEC and HrAAEH groups showed normal histology.
Figure 2: Histopathological images of renal tissue sections of NORM group, untreated HyGR (DIAB) and HyGR treated with 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) 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. The NORM and HrAGHR groups showed normal histology.
The SCC of the DIAB group was significantly ($p < 0.05$) higher than that of the NORM group (Figure 3). SCC of HrACMO = 0.58 ± 0.05 mg/dL was significantly ($p < 0.05$) lower than the NORM = 0.71 ± 0.02 mg/dL. Generally, HyGR treated with SHfs gave SCCs in the following order: HrHIRO = 0.89 ± 0.04 mg/dL > HrEMCO = 0.77 ± 0.03 mg/dL > HrASGA = 0.70 ± 0.03 mg/dL > HrACMO = 0.58 ± 0.05 mg/dL. HyGR treated with DHfs gave SCCs that were relatively within a narrow range of 0.61 ± 0.02 mg/dL – 0.78 ± 0.03 mg/dL. SCC of HrAMAE = 0.69 ± 0.04 mg/dL was comparable with the NORM = 0.71 ± 0.02 mg/dL, whereas HrAMAH = 0.84 ± 0.05 mg/dL and HrAMEH = 0.80 ± 0.03 mg/dL indicated significant ($p < 0.05$) elevation of SCC. The SCCs of HyGR treated with THfs were not significantly ($p > 0.05$) different from that of the NORM group.

Figure 4 showed that SUC of the DIAB group was significantly ($p < 0.05$) elevated than that of the NORM group. The SUCs of HyGR treated with DHfs did not show significant ($p > 0.05$) variability (61.7 ± 16.3 mg/dL – 62.3 ± 14.3 mg/dL); except HrHIRO = 53.0 ± 13.3 mg/dL. Comparative analyses showed that SUCs of HrAGAM and HrAGEC were amongst the lowest values of SUCs, which corresponded to 3.28 and 3.83 folds lower than that of the DIAB group respectively. However, SUCs of HrAGHR and HrAMHR were significantly ($p < 0.05$) elevated compared with that of the
NORM group, whereas HrAMEC = 41.1 ± 10.2 mg/dL and HrECHR = 53.2 ± 11.9 mg/dL were lower than that of the NORM group. Although SUC of HrAGEH = 76.8 ± 19.0 mg/dL was significantly \( (p < 0.05) \) elevated compared with the NORM = 58.2 ± 12.3 mg/dL; the value represented 0.10% lower than SUC of the DIAB group. Also, SUCs of HrAMAE, HrAMAHE and HrAMEH were lower than that of the NORM group.

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

Serum AST, ALT and ALP activities of the DIAB group were significantly \( (p < 0.05) \) higher than that of their corresponding NORM groups (Figure 5). Serum AST of HyGR treated with SHfs showed lower levels of enzyme activities than that of the NORM group. Specifically, serum AST activities of HyGR treated with SHfs were within the range of 12.3 ± 3.4 IU/L – 16.6 ± 5.6 IU/L. Serum AST activity of HrAGHR = 22.1 ± 2.3 IU/L and HrAMEC = 24.7 ± 3.1 IU/L were comparable with that of the NORM group \( (p > 0.05) \), whereas HrAGEC = 16.8 ± 1.9 IU/L, HrAMHR = 15.6 ± 3.6 IU/L and HrECHR = 19.2 ± 3.4 IU/L were significantly \( (p < 0.05) \) lower than the NORM group. Amongst the HyGR treated with THfs, serum AST activity of HrAMAH = 9.8 ± 1.3 IU/L gave the lowest enzyme activity. Serum AST activities of HrAGEH, HrAMAE and HrAMEH were within the range of 13.3 ± 1.8 IU/L – 15.7 ± 3.8 IU/L. Serum AST activity of HrAAEH = 14.1 ± 1.1 IU/L corresponded to 70.3% reduction in AST activity relative to the DIAB group.

Serum ALT activity of the DIAB group was significantly \( (p < 0.05) \) higher than that of the NORM group (Figure 5). Serum ALT activity of HyGR treated with SHfs exhibited a narrow variability that was within the range of 9.0 ± 8.1 IU/L – 11.9 ± 2.4 IU/L. Also, Figure 5 showed that serum ALT activity of HrAGAM = 7.1 ± 1.3 IU/L, HrAMHR = 7.9 ± 4.2 IU/L and HrECHR = 6.9 ± 2.1 IU/L were lower than that of the NORM group; \( p > 0.05 \). HrGR treated with THfs gave serum ALT activity in the following order: HrAMEH = 14.7 ± 1.7 IU/L > HrAMAE = 13.2 ± 4.2 IU/L > HrAMAH = 11.7 ± 1.0 IU/L > HrAGEH = 7.1 ± 2.3 IU/L. Finally, serum ALT activity of HrAAEH = 8.4 ± 1.4 IU/L was not significantly different \( (p > 0.05) \) from the NORM = 8.47 ± 3.3 IU/L.

Figure 5 showed that serum ALP activity of the DIAB group was significantly \( (p < 0.05) \) elevated than that of the NORM group. Similarly, serum ALP activity of HyGR treated with SHfs were higher than that of the NORM group, except HrEMCO = 33.2 ± 5.2 IU/L; NORM = 38.8 ± 8.3 IU/L. Serum ALP activity of HrAGAM = 85.5 ± 19.3 IU/L was higher than the DIAB = 81.4 ± 4.1 IU/L; \( p < 0.05 \), whereas serum ALP activity of HrAMEC = 34.9 ± 3.1 IU/L, HrAMHR = 32.1 ± 4.3 IU/L and HrECHR = 31.6 ± 4.8 IU/L were comparable with that of the NORM group. An overview of Figure 5 showed that ALP activity of HrAAEH = 56.1 ± 19.3 IU/L was higher than the NORM group; \( p < 0.05 \).
The STBC of the DIAB = 13.2 ± 2.6 mol/L was not significantly (p > 0.05) higher than that of the NORM = 10.7 ± 1.2 mol/L (Figure 6). Furthermore, STBC of HyGR treated with SHFs were within narrow range of 12.5 ± 1.3 mol/L – 13.5 ± 0.9 mol/L and comparable with STBC of the DIAB group. STBC of HrAGHR, HrAAEH and HrAMHR were equivalent to that of the DIAB group. Finally, STBC of HyGR treated with THfs were generally lower than that of the DIAB group; p > 0.05.

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. For instance, according to Koneri et al., saponin isolated from Momordica cymbalaria Fenzl caused considerable quantitative increase and rejuvenation of β-cells (75%) of streptozotocin induced diabetic rats, in which oleane-type triterpenoid saponin was implicated as the active anti-diabetic principle of the plant extract. Another study by El-Soud et al., 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 HyGR. 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 HrASGA, HrAGHR, HrAMHR groups (Figure 1) and HrAGEC and HrAAEH groups (Figure 2).

There are established evidence in support of the fact that structural distortions and damage to tissues of diabetic animals are outcome of excessive production of free radicals and oxidative stress. 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 herbal 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.

Elevations of blood creatinine and urea concentrations are diagnostic of renal dysfunction and creatinine concentration parallels that of urea. Therefore, pathologic conditions that
impair renal function cause raised blood creatinine and urea levels. It’s worthwhile to note that raised blood creatinine and urea levels are outcome of longstanding DM complications leading to diabetic nephropathy.\textsuperscript{66-68} However, using clinical survey protocol, Hjelmesath \textit{et al.},\textsuperscript{69} 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 reports\textsuperscript{70,71}, 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 HyGR of the following categories: HrASGA, HrAGHR, HrAMHR, HrAGEC and HrAAEH. Related studies have also established the capability of herbal extracts of \textit{Momordica charantia}\textsuperscript{48} and \textit{Vernonia amygdalina}\textsuperscript{45} to rejuvenate and protect renal tissues of alloxan induced diabetic mice.

Nannipieri \textit{et al.},\textsuperscript{72} 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, which was in concord with the previous suggestions of Henderson \textit{et al.},\textsuperscript{73} and subsequent findings of Fraser \textit{et al.}\textsuperscript{74} Also, West \textit{et al.},\textsuperscript{75} reported incidence of raised levels of serum ALT activity in Type 1 and Type 2 DM. According to Nannipieri \textit{et al.},\textsuperscript{72} mild elevations of hepatic enzymes occurred in plasma of DM patients; of which only raised γ-glutamyl transferase (GGT) activity was an independent predictor of impaired glucose tolerance, especially in Type 2 DM. Additionally, the use of ALP isoform band 10 (ALP-10) in the screening of autoimmune disorders, as in the case of Type 1 DM, has been described elsewhere.\textsuperscript{76} 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. Raised level of serum ALP activity supports the metabolic etiology of bone disease in DM.\textsuperscript{77-79} Although the absolute level of activity of serum AST > ALT\textsuperscript{80} 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 non-alcoholic fatty liver disease (NAFLD)\textsuperscript{79,80,83} as corroborated by the present findings (Figure 1), which was a classical Type 1 DM prototype, and in Type 2 DM.\textsuperscript{74,85} The results of the present study showed evidence of raised levels of hepatic enzymes (AST, ALT and ALP\textsuperscript{86}) activities in serum of untreated HyGR when compared with those treated with the various herbal formulations. Therefore, the experimental herbal extracts displayed varied capacities to ameliorate hepatic injury in HyGR.

The relatively higher serum bilirubin concentration of untreated HyGR (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\textsuperscript{87,90}, rejuvenates of endothelial function in Type 2 DM\textsuperscript{91} and suppresses the free radicals induced peroxidation of lipids and lipoproteins.\textsuperscript{92} Specifically, reports showed that higher serum bilirubin levels, within normal range, might be predictive of lower risk of progression towards nephropathy in Type 2 DM.\textsuperscript{90,93} It thus appears that serum bilirubin levels fulfill dual criteria for clinical diagnosis and physiologic therapeutic intervention strategy.

\textbf{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, AGEC and AAEH herbal formulations exhibited hepatic protective effects, whereas ASGA, AGHR and AMHR herbal formulations ameliorated renal tissue injuries in HyGR.
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