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Research Article
Synergistic Anti-diabetic Activity of *Gongronema latifolium* and *Telfairia occidentalis* Leaves Extracts on Hepatic Function and Hematological Indices in Wistar Rats

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Abstract

Background and Objective: Persistent hyperglycaemia in diabetes mellitus (DM) could result to compromised hepatic function. *Telfairia occidentalis* (TEL) and *Gongronema latifolium* (GON) have both demonstrated glucose lowering potentials in previous investigations due to a rich mix of bioactive compounds in the plants. This study investigated the synergistic antidiabetic and hepatoprotective potentials of TEL and GON in diabetic rats. **Materials and Methods:** Six groups of animals were studied; normal control (NC), diabetic control (DC), metformin control (MET), TEL and GON groups, single and combine (n = 7). NC and DC received placebo treatments, other treatment groups were given 500 mg kg^{-1} b.wt. doses of the extracts and MET for 21 days. The rats were sacrificed, blood and liver tissues collected for hepatic enzymes, haematological and histo-pathological evaluation. **Results:** Transferases (aspartate-AST and alanine-ALT), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) increased in DC rats compared to NC ($p<0.05$). Treatment significantly decreased ($p<0.05$) the enzyme concentrations in TEL/GON treatment commensurate to standard MET. Haematological indices, white blood cells count (WBC) and its differentials lymphocytes (LYM) and monocytes (MON) were significantly increased ($p<0.05$) in DC compared to NC. Red blood cells (RBC) and platelets concentrations which decreased in DC were significantly increased ($p<0.05$) in all treatments and compared significantly to MET. Histo-pathological evaluation of the liver showed significant regeneration across all treatments with more pronounced effect in TEL/GON group. **Conclusion:** These findings reaffirmed the hypoglycaemic potentials of TEL and GON, suggesting these plants in synergism may potentiate a better hepatoprotective and increased antidiabetic activity.

Key words: *Telferia occidentalis*, *Gongronema latifolium*, hepatic function, hematological indices, diabetes mellitus

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a household name for a condition which alters the body's capacity to perfectly utilize the energy found in food[1]. The trademark of this derangement is the inability of the affected individual to effectively control blood glucose[2]. This actually takes place due to either the pancreas not being able to produce sufficient insulin or the unresponsiveness of cells to insulin available[2]. As such, two clinical syndromes ensue: first is described by insulin reliance and premature time of onset with decrease in body mass and ketonuria and the other one described by generally late occurrence, insensitivity to insulin and incomplete insulin shortage[3].

This is of serious public health concern and in the last few decades, diabetes has become a serious disease with epidemic status in the global scene[4]. From the Centre for Disease Control speculations[5], diabetes in the urban populace in developing countries will double by 2030 following the current trend. These speculation shows that the "diabetes epidemic" will continue to increase, although this might likely be an underestimation of future diabetes prevalence. With this growing concern of diabetes, organs and tissues involved in metabolism are exposed to loads of unpleasant reactive species with attendant consequences of toxicity and cellular damage. The liver is the most prone organ to metabolic derangement; it is considered one of the most vital organs in the biological system basically because of the huge role it plays in metabolism of virtually all biomolecules in addition to other numerous biochemical functions[6]. The functioning of the liver cells automatically alters when it is exposed to several chemical substances and this may be accompanied by pathological alteration seen in most liver diseases example liver cell damage and cholestasis. However, biochemical examination of liver cell dysfunction such as measurement of serum enzyme can make available proof of hepatocellular injury[7].

Telfairia occidentalis commonly known as "fluted pumpkin" is a seed and leave vegetable that is common in the West and Central Africa specifically in the forest zones, most commonly in Cameroon, Benin and Nigeria[8]. The plant is well- known and a highly consumed vegetable all over Nigeria[9]. Earlier studies on *T. occidentalis*[10] revealed that it is a good dietary source of carbohydrate, protein, fat and oil and dietary fibres. The study also indicated that *T. occidentalis* is rich in mineral element. Christian[11] indicated that *T. occidentalis* has a high content of vitamin A and other vitamins like nicotinamide, thiamin, riboflavin and ascorbic acid, as well as amino acids such as glycine, alanine, histidine, aspartate, glutamine, lysine, methionine, tryptophan, threonine, serine, phenylalanine and valine. This myriad of nutrient composition makes *T. occidentalis* one of the most important green vegetables consumed by man.

The fresh leaves can be cut into tiny pieces, then mixed properly with salt and coconut water, kept in a well cocked container and used after some hours for the management of convulsion in ethno-medicine[12]. This leaf is also effective in the treatment of hypercholesterolaemia, liver complication and deteriorated immune system[13]. Previous studies by Kayode *et al*.[14], Adaramoye *et al*.[15] and Oboh *et al*.[16] have all reported the free radical scavenging potential and antioxidant constituent in *T. occidentalis*.

Gongronema latifolium is an herbaceous shrub, with light green rope-like stem, which yields characteristic milky exudates[17]. *Gongronema latifolium*, is generally trusted to have important nutritional values. This vegetable is highly utilizable in many ways, in different places to prepare delicacies such as fresh fish oil soup, salad[18]. Although information on potential food uses of *Gongronema* is quite scanty, proximate analysis of this vegetable has shown that it is a valuable source of proteins, fat and oil and vitamins as well as carbohydrates and dry matter. Eleyinmi[19] reported a study on the chemical composition of *G. latifolium* that the vegetable is rich in mineral elements: potassium, sodium, calcium, phosphorus and cobalt with varying levels of essential amino acids.

Gongronema latifolium being one of the most extensively studied greenly vegetable has been reported to have medicinal relevance especially in the folk medicine[19] Antioxidant potential of this plant has been reported by Atawodi[20], anti-inflammatory properties[21-23], anti-bacterial activity[19], hypoglycaemic and hypolipidaemic potentials[18]. All these medicinal attributes makes *G. latifoliiim* unquestionably essential and vitally important for the maintenance of good health.

There is nevertheless no substantial information on how these vegetables, in a combined state, elicit their actions. Because of the health concern of the people consuming these medicinal herbs in a combined state, this study took a step further in investigating the positive synergistic effect of combined crude extracts of *Gongronema latfolium* and *Telfairia occidentalis* in diabetic rat models.

MATERIALS AND METHODS

Material/apparatuses: This study was carried out in the last quarter of 2018, in the Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Nigeria.

Manual blender, weighing balance, separating funnels, needle and syringe, filter papers, chess cloth, rotary evaporator, refrigerator, water bath, foil paper, Mind ray auto-haematology analyzer, centrifuge, dissecting set, one touch glucometer, measuring cylinder, beakers.

Chemicals and solvents: All chemicals and reagents used were of analytical grade. The chemicals/reagents used in the study were purchased from Calbiochem (an affiliate of Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich, except otherwise stated. Dichloromethane (JHD), ethanol (BDH), chloroform, sodium citrate, dimethylsulfoxide, streptozotocin.

Collection and authentication of plants material: The leaves from *Gongronema latifolium* and *Telfairia occidentalis* were obtained from Akamkpa in Cross River State, Nigeria in the early hours of the day. Plant authentication was done by a botanist in the Department of Botany, University of Calabar. They were properly rinsed to remove sand and dirt and then air-dried for 7 days. The dried leaves were crushed separately using laboratory ken-wood blender. The powdered leaves were kept in glass bottles (separately) with a plastic screw cap and preserved in a refrigerator at 4°C.

Preparation of plant extract: Dichloromethane and ethanol in the ratio of 1:1 was used for the extraction of the blended powdered leaves. Extraction was done by the method of cold maceration which was done by soaking the blended leaves in the solvent (a mixture of dichloromethane and ethanol) separately for 48 h, with intermittent stirring to allow for thorough mixing. The mixture was thereafter filtered first with chess cloth then with filter paper (Whatman No. 2 filter paper). Concentration of extracts was done using rotary evaporator (Laborota 300 Resona). The filtrate (extract) was concentrated under reduced pressure at 50°C in rotary evaporator to 10% volume and then to absolute dryness with the help of water bath. The total yield of 47.8 g was obtained from each plant per 1000 g of the leaves extracted. The extracts were kept in a beaker, stored in refrigerator until it was needed for the treatment.

Experimental design: A total of 42 mature albino Wistar rats of different sexes, weighing between 120-200 g were bought from the animal house, Biochemistry Department, University of Calabar and used for this experiment. The rats were kept in well ventilated wooden cages with a wired mesh top under normal tropical room temperature and relative humidity. The 42 rats were divided into 6 groups of 7 rats as arranged in Table 1. This procedure was in line with the guidelines of the National Institute of Health (NIH)[24] for laboratory animal care and use.

Induction of experimental diabetes: Diabetes was induced in the experimental rats after a 24 h fast[25] with 40 mg kg^{-1} b.wt., STZ using 0.5 M sodium citrate buffer reconstituted in dimethylsulfoxide. Their fasting blood glucose and the body weight was properly measured. The induction was done intra-peritoneally and after 72 h of induction, diabetes was confirmed with animals having fasting blood glucose (FBG) concentration of $\geq$200 mg dL^{-1} using one touch glucometer.

Toxicity testing: Toxicity studies to determine the minimal lethal dose (LD_{50}) of the combine plant extracts was performed using experimental mice obtained from Pharmacology Department, University of Calabar following the method described by Lorke[26]. The combine extracts were safe up to 5000 mg kg^{-1} b.wt.

Treatment procedure: The diabetic and non-diabetic albino Wistar rats were treated as follows: The normal control group was given placebo orally, the *G. latifolium* group, *T. occidentalis* and the combined groups were treated orally with 500 mg kg^{-1} b.wt., of the extract as determined from previous studies. The standard drug group (Metformin) was given 500 mg/70 kg b.wt. Treatment lasted for 21 days and throughout this period animals were maintained with animal feed (pallets) manufactured by Vital Feeds, Jos, Plateau State and tap water both *ad libitum*.

Table 1: Experimental grouping of the animals, treatment and feeding

Groups	Number of rats	Treatment/feeding
Normal control	7	Normal diet: Distilled water and animal feed
Diabetic control	7	Normal diet: Distilled water and animal feed
Gongronema group	7	Crude extract from *G. latifolium* only+animal feed
Telfairia group	7	Crude extract of *T. occidentalis*+animal feed
Combined group	7	Combined crude extract of *G. latifolium* and *T. occidentalis*+animal feed
Metformin group	7	Metformin treatment+animal feed

Fasting blood glucose and body weight measurement: Fasting blood glucose of the experimental rats were tested at interval of 3 days using a one touch® glucometer while the body weight were measured also at every 3 days using bench top digital balance.

Collection and preparation of tissues and analysis of blood: After the 21 days treatment, the rats were fasted overnight and their weights were taken again before they were anaesthetized with chloroform vapour and dissected. Whole blood was collected by cardiac puncture into plain specimen bottles for varied biochemical analysis. One lobe of the liver was also harvested for histo-pathological studies. After standing for about 2 h, the now coagulated blood samples were centrifuged at 3000 rpm for 10 min using an MSE table top centrifuge and serum collected using a semi-automatic pipette into labeled specimen tubes and preserved in the refrigerator till when required for biochemical analysis.

Determination of indices of hepatic function: Activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined using Randox Laboratory kits (Randox Laboratories, UK) method[27]. The method of Tietz[28] was employed in the alkaline phosphatase, (ALP) estimation. Gamma glutamyl transferase (GGT) activity was assayed using Agappe based on Szasz[29].

Hematological and histological studies: Hematological analysis was done using auto hematology analyzer BC-2800 from Mindray while histological examination of the liver tissues was done according to the method of Jarrar and Taib[30] using haematoxylin and eosin staining techniques. Briefly, fixed tissue (liver) sections were processed following the under listed procedures; the tissues were washed in tap water for 30 min, dehydrated, cleared, impregnated, embedded, sectioned and stained with haematoxylin for 30 min and then washed in tap water. This was followed by differentiation with 1% acid alcohol until correct differentiation was clear under the microscope. The specimens were counterstained in 1% eosin for 1 min and washed in running water to remove excess eosin. The specimens were again dehydrated by passage through a series of graded concentrations (50, 70 and 90%) of ethanol for 1 min each. The specimens were cleared in xylene and mounted using a drop of Canada balsam on the specimens and covered with the glass cover slips for study under the microscope.

Statistical analysis: Data obtained was analyzed by one way analysis of variance (ANOVA) using SPSS package version 20 and Turkey *post hoc* multiple range test. All data was expressed as Mean±SEM (n = 7) and hypothesis tested at 95% level of significance.

RESULTS

Effect of treatment on body weights: Body weights of the diabetic untreated group (DC) were consistently observed to drop through the period of the experiment which was a clear opposite to the increased weight in normal control group as rats in this group showed steady weight gain through the experimental period (Fig. 1). Amongst the diabetes induced treatment groups were different degrees of weight changes with all extract treated groups showing an initial loss in weight which was followed by slight weight gains. The extract treatments, although were not as effective as the standard anti-hyperglycemic drug-metformin in improving the body weight, still showed potentials at preventing the drastic weight loss observed in the untreated diabetic group. The combined treatment groups were observed to have closest results to that of the synthetic drug (metformin) treated group especially in the first 2 weeks of the study, although like every other treatment group, the group showed an initial lag in weight values and the weight gains were subsequently significant when compared to the diabetic control.

Effect of treatment on hematological indices: Hematological indices assayed for included white blood cells (WBC), red blood cells (RBC), haemoglobin concentration (HGB), haematocrit (HCT), platelets (PLT), lymphocytes (LYM) and monocytes (MON) (Table 2). The WBC count showed a significant ($p<0.05$) elevation in concentrations in the diabetic control (10.92±1.45) when compared with the concentration in the normal control (7.80±0.66). Although there was reduction in WBC in all the treatment groups, MET and combine treatment groups also showed significant decrease (7.48±0.50 and 8.16±0.35, respectively) in WBC when compared to the diabetic control. The TEL treatment had a WBC concentration (10.52±1.01) which was similar to that of the diabetic control and significantly higher ($p<0.05$) than that of the normal control. Red blood cell concentrations were quite reduced in the diabetes control groups (5.02±0.07) when compared to that of the normal control. GON treatment group showed significant increase as well when compared with the diabetic control group (5.02±0.07)

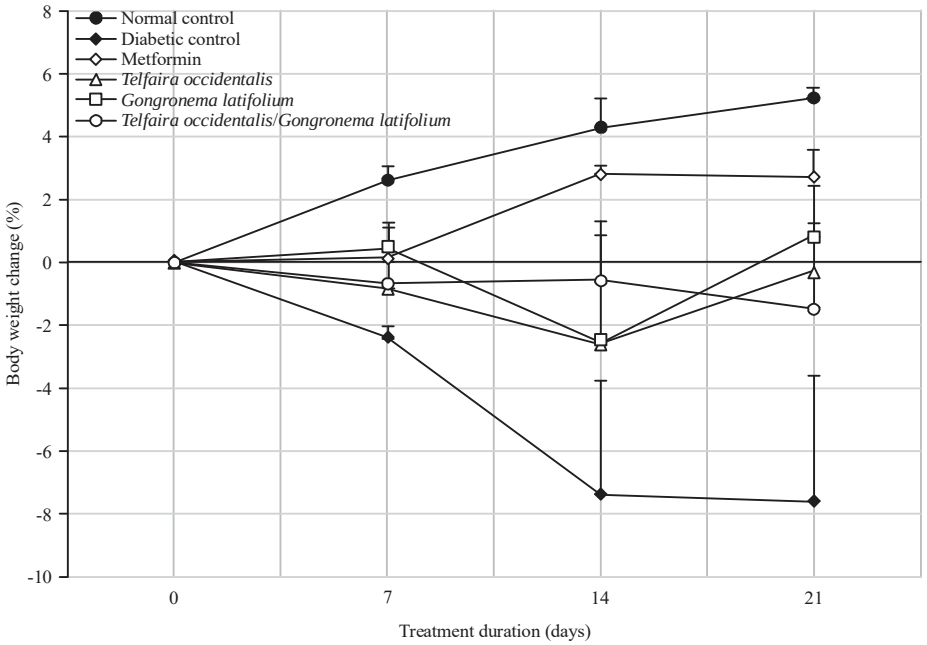

Fig. 1: Mean percentage change in body weight of the different experimental groups
Values expressed as Mean±SEM, n = 7

Table 2: Effect of treatment of *T. occidentalis*, *G. latifolium* and combined crude extracts on some haematological indices in the different experimental groups

Groups	WBC ($\times 10^3$ cells μL^{-1})	RBC ($\times 10^6$ cells μL^{-1})	HGB (g dL^{-1})	HCT	PLT ($\times 10^3$ cells μL^{-1})	LYM (%)	MON (%)
NC	7.80±0.66	6.35±0.12	10.50±0.30	41.97±1.42	611.75±69.53	72.77±4.97	5.16±0.80
DC	10.92±1.45*	5.02±0.07	10.85±0.20	43.25±0.09	415.00±41.57*	102.15±3.53	10.25±2.04
MET	7.48±0.50[a]	6.82±0.31	11.27±0.44	43.97±1.63	505.26±21.73	77.77±3.07	8.67±1.55
TEL	10.52±1.01*[,b]	6.66±0.20	11.33±0.18	44.78±1.04	405.25±.26.71*[,a]	81.95±1.21[a]	10.57±1.22*
GON	9.18±0.67	7.02±0.19[a]	12.30±0.30*[,a,b,c]	48.15±1.26*[,b]	476.0±52.19*	84.10±1.81*[,a]	10.16±1.00*
TEL/GON	8.16±0.35[a,c]	6.28±0.16	10.62±0.35[b]	39.23±1.93[b,c,d]	463.00±28.37*	83.12±3.88*[,a]	8.67±2.56

Values are expressed as Mean±SEM, n = 7, *Significantly different from NC at p<0.05, [a]Significantly different from DC (diabetic control) at p<0.05, [b]Significantly different from MET at p<0.05, [c]Significantly different from TEL at p<0.05, [d]Significantly different from GON at p<0.05, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*

whilst there was no significant different (p<0.05) in TEL/GON group (6.28±0.16) when compared to the diabetic control and the GON treatment group. Haemoglobin concentration was increased significantly (p<0.05) in only the GON treatment (12.30±0.30) when compared to DC (10.85±0.20) and NC (10.50±0.30). Haematocrit results showed that GON treatment group had elevated levels of HCT (48.15±1.26) which was significantly increased (p<0.05) when compared to the normal control and MET groups. The TEL/GON combined treatment had HCT concentrations (39.23±1.93) which was significantly lowered (p<0.05) when concentrations were compared to the MET, TEL and GON treatments. Platelet concentration were significantly decreased (p<0.05) in all diabetic treated groups with exception of the MET group (505.25±21.73) when compared to NC (611.75±69.53). Differential WBC count for lymphocytes and monocytes were also assayed. LYM assay showed all diabetes treated groups to have increased LYM levels when compared to the normal control (72.77±4.97). However, only those of the TEL, GON and TEL/GON treatment groups (81.95±1.21, 84.10±1.81 and 83.12±3.88 respectively) were observed to be significantly decreased (p<0.05) when compare to the diabetic control (102.15±3.53). Monocyte concentrations were also observed to increased significantly (p<0.05) in the diabetic control, TEL and GON treatments (10.25±2.04, 10.57±1.22 and 10.16±1.00) when compared to the normal control (5.16±0.80). However, MET (8.67±1.55) and the combined group (8.67±2.56) showed significant drop in the levels of monocyte at the end of the experiment.

Effect of treatment on blood glucose concentration of different groups: The effect of treatment on different experimental groups is as shown in Fig. 2. Indicating line plots of the average percentages in serum glucose concentration in

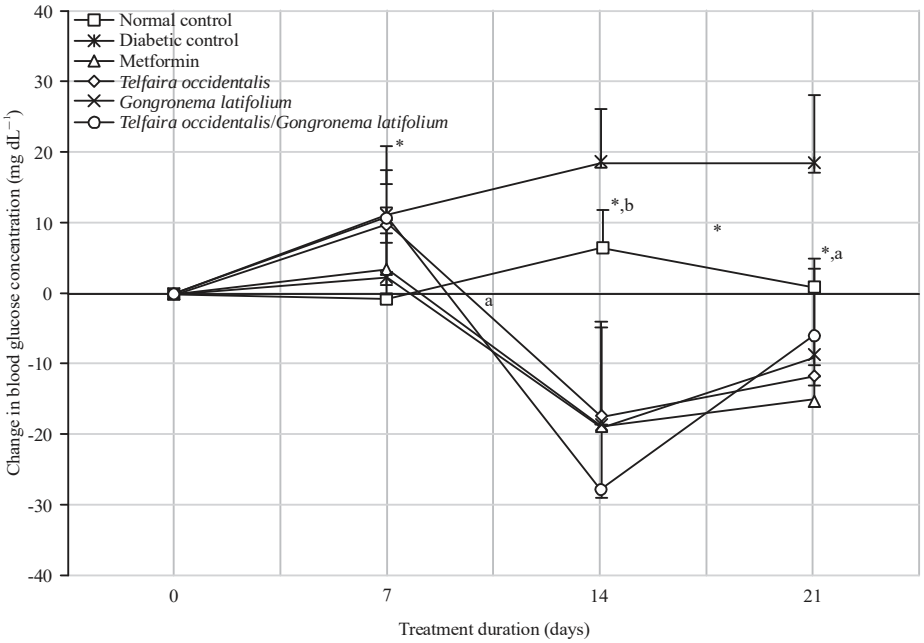

Fig. 2: Percentage changes in fasting blood glucose concentration for the different experimental groups
Values are expressed as Mean±SEM, n = 7, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*, *Significantly different from NC at p<0.05, [a]Significantly different from DC at p<0.05, [c]Significantly different from MET at p<0.05

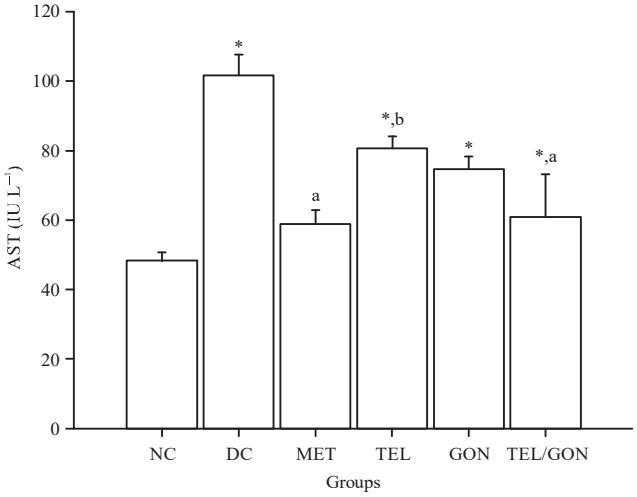

Fig. 3: Comparison of aspartate aminotransferase concentrations in the different experimental groups
Values are expressed as Mean±SEM, n = 7, *Significantly different from NC at p<0.05, [a]Significantly different from DC at p<0.05, [b]Significantly different from MET at p<0.05, [c]Significantly different from TEL at p<0.05, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*

the experimental groups following treatment. There was initial increase in serum glucose concentrations in all diabetes induced groups and this was significant compared to normal control (p<0.05). Observation in the weeks that followed showed declining blood glucose levels for the diabetic groups being treated and this was significant compared to DC (p<0.05). These decline varied between 11 and 22% in the different experimental groups with the maximum percentage decreased being observed in the combined group (Tel/Gon = -27.82%). The diabetic untreated group showed further increase having a cumulative increase of about 18.66%. During 14-21 days of the experiment, slight increase in blood glucose concentrations in the diabetic treated groups were observed whilst the diabetic untreated group showed slight decrease (about 0.01%) through the experimental period, the normal control had values within physiological range varying only slightly.

Effect of treatment on selected liver enzymes: Serum aspartate amino transferase (AST), serum alanine amino transferase (ALT), serum alkaline phosphatase (ALP) and serum gamma glutamyl transferase (GGT) concentrations were as recorded in Fig. 3-6. Serum AST concentrations were significantly (p<0.05) elevated in all treatment groups when compared to concentrations of the normal control (48.12 µL). Results showed that diabetic control (DC) had values which were highest (101.57 µL) and compared significantly with normal control and

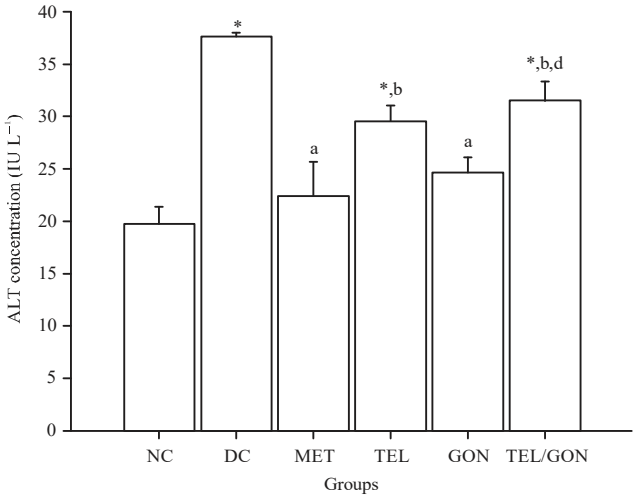

Fig. 4: Comparison of serum alanine aminotransferase concentrations in the different experimental groups
Values are expressed as Mean±SEM, n = 7, *Significantly different from NC at p<0.05, [a]Significantly different from DC at p<0.05, [b]Significantly different from MET at p<0.05, [d]Significantly different from GON at p<0.05, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*

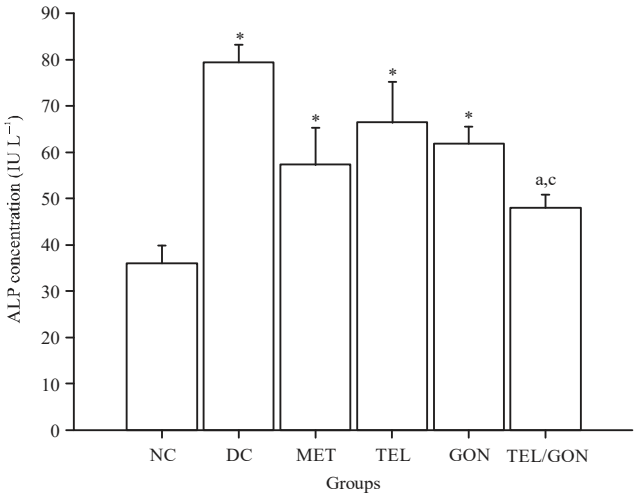

Fig. 5: Comparison of alkaline phosphatase concentrations in the different experimental groups
Values are expressed as Mean±SEM, n = 7, *Significantly different from NC at p<0.05, [a]Significantly different from DC at p<0.05, [c]Significantly different from TEL at p<0.05, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*

treatment groups (p<0.05). Serum alanine amino transferase (ALT) concentration assayed showed significantly increased (p<0.05) in the diabetic control group (35.57 µL) (p<0.0%), when compared to normal control (19.76 µL), TEL

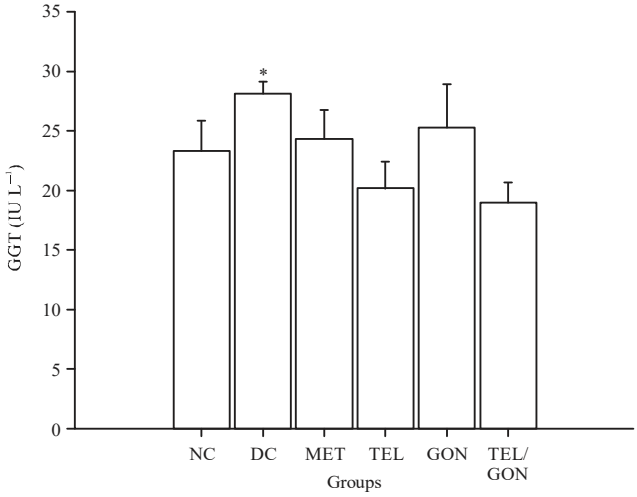

Fig. 6: Comparison of gamma glutamyl transferase concentrations in the different experimental groups
Values are expressed as Mean±SEM, n = 7, *Significantly different from NC at p<0.05, NC: Normal control, DC: Diabetic control, MET: Metformin standard control, TEL: *Telfaira occidentalis*, GON: *Gongronema latifolium*

(29.55 µL) and TEL/GON (31.47 µL) combined treatment groups however showed slight reduction in ALT level when compared to metformin and the GON group (22.38 and 24.65 µL, respectively). Serum alkaline phosphatase (ALP) concentrations in all treatment groups (with the combined group being an exception) were increased significantly (p<0.05) when compared to the normal control. The TEL/GON combined treatments showed significant increase (p<0.05) in ALP concentration (48.32±27) when compared to the diabetic control (79.46±3.73) and the TEL treatment groups (66.45±8.87). Serum gamma glutamyl transferase (GGT) concentration was not significantly affected (p<0.05) in the various treatments with the only exceptions in DC and MET treatments.

Effect of treatment on the histology of the liver architecture: Section of liver tissues show a regular architecture; a prominent central vein (CV) and hepatocytes (HP) radiating outwards. The hepatocytes have deeply stained nuclei and clear cytoplasm. The sinusoidal spaces (SS) are dilated and the portal tracts have an intact limiting plate and consist of the bile duct, hepatic artery and portal vein in the normal control animals plate. In Fig. 7; the diabetic rats showed congested central vein (CV) with radiating plates of swollen hepatocytes (HP) showing prominent nucleoli and separated by dilated sinusoidal spaces (SS) with the portal areas showing an irregular limiting

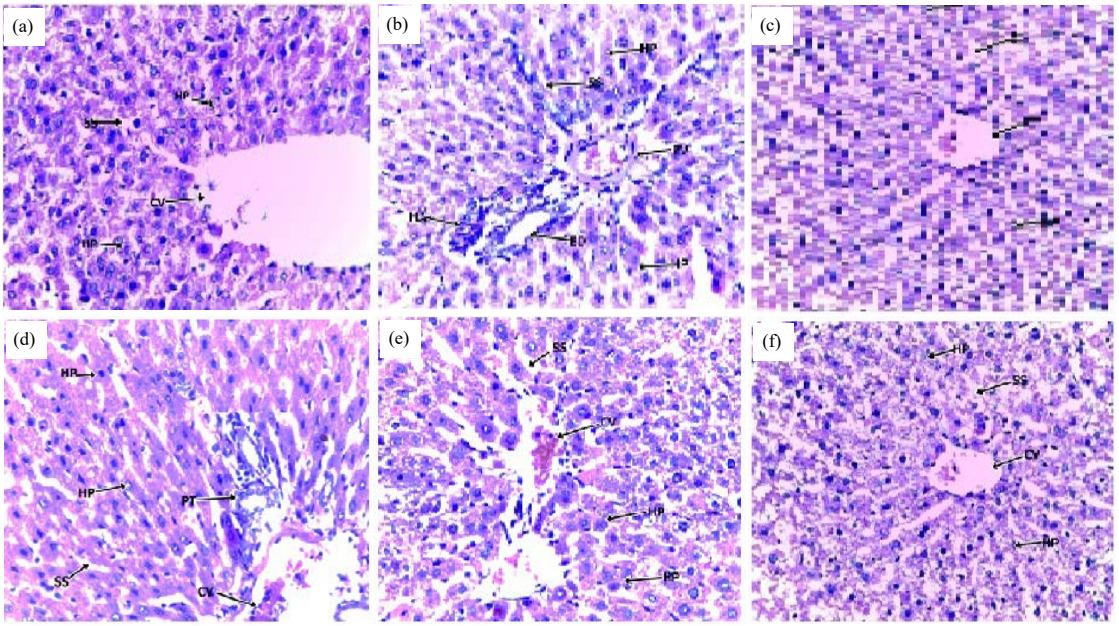

Fig. 7(a-f): Histological examination of the liver, Photomicrograph of (a) Normal control rat liver treated with placebo, (b) Diabetic rat treated with placebo, (c) Treated with metformin, (d) Treated with *G. latifolium*, (e) Treated with *T. occidentalis*, (f) Treated with combined extracts of *T. occidentalis* and *G. latifolia* (Mag. X400)
CV: Central vein, HP: Hepatocytes radiating outwards, SS: Sinusoidal spaces, PV: Placebo portal vein, HA: Hepatic artery, BD: Bile duct

plates of hepatocytes with portal vein (PV), hepatic artery (HA) and bile duct (BD). In sum these abnormalities in the liver architecture of the diabetic rats were restored in all treatments (Fig. 7a-f).

DISCUSSION

This study evaluated the effect of *Gongronema latifolium* and *Telfairia occidentalis* leaves extract in single and combined form on hepatic enzymes and hematological indices in STZ induced diabetes in Wistar rats. Although oral hypoglycemic agents and insulin have been substantially efficient in the management of diabetes and regulation of high blood sugar, there have been noticeable adverse effects[31]. An alternative has been the use of medicinal plants which have shown great potentials in the management of hyperglycaemia with minimal complications[32]. The utmost value of such medicinal plants being the bioactive components with therapeutic importance (including lowering high blood sugar levels). The adverse effects of diabetes has been known to affect a number of internal organs one of such being the liver such as those observed by Mohamed *et al.*[33].

Experimentally, STZ has often been used to induce this complication[34]. The hyperglycaemia was as a result of the non-production of insulin occasioned by the damage of the beta cells within the pancreas which are accountable for the production of insulin. Treatment using the varied extract formulations and the standard oral antidiabetic drug-metformin did not bring about an immediate recovery during the 1st week of the experiment. However, there was significant decline in the percentage blood glucose concentrations of the treated groups in the 2nd week post treatment. Earlier studies with these plants (*G. latifolium* and *T. occidentalis*)[35] revealed a significant presence of alkaloids, glycosides, saponins, flavonoids, steroids, terpenoids and phenol in both plants. Hence, the observed modulation of serum glucose on treatment with the extracts from these plants could be attributable to these rich phyto-principles. Saponins have been reported to stimulate the secretion of insulin, beta cells regeneration in the islets and enhance the activity of enzymes responsible for glucose utilization[36]. Saponins are also be strongly involve in blood glucose regulation via different mechanisms[36], hence, a major factor in the anti-diabetic potentials of these plants.

As a known complication associated with diabetes, mean body weights change (%) within the experimental period showed severe negative fluctuations with exception of the normal control group. The declining body weight is attributed to an increased utilization of the body energy

reserve occasioned by the absence of the regulatory hormone insulin[36]. Other factors including water loss, hyperosmolarity due to depleted intercellular water thereby stimulating polydipsia in diabetes could also be a factor[36]. Body weights for the diabetic control group (DC) were consistently observed to decrease significantly (up to -7.62%) through the period of the experiment when compared to the treated groups which showed varied degrees of recoveries. Worthy of note is the results obtained from the metformin treated group and combined group; although the synthetic drug elicited a better effect when compared to all the treated groups, the combined treated group also showed a significant increase in bodyweight especially in the second and third week of the experiment.

Outcome of this study showed significant elevation ($p<0.05$) in serum concentrations of all the hepatic enzymes (AST, ALT, ALP and GGT). Gamma-glutamyl transferase (GGT) concentration was significantly ($p<0.05$) elevated in the serum of diabetic control group when compared alongside ALP, a strong indication of liver damage in diabetes. Chronic elevations in transaminases (AST and ALT), alkaline phosphatase and gamma-glutamyl transferase are common occurrences in diabetic conditions[33].

A peripheral WBC count is reported to be connected with insulin resistance[37], coronary heart disease[38] and diabetes micro and macrovascular complications[39]. Hence the observed significantly ($p<0.05$) elevated levels of WBC seen with haematological indices in the DC control. Differential WBC count for lymphocytes showed increased level in diabetic control group which was significant ($p<0.05$) compared with the normal control.

The incidence of anaemia in diabetic patients has been reported to occur as a result of elevated non-enzymatic glycosylation of RBC membrane proteins, which correlates with high blood glucose[40]. Oxidation of the proteins and hyperglycaemia in diabetes mellitus leads to an elevated production of lipid peroxides which automatically causes the haemolysis of RBC[41]. Hence, lowered levels of RBC observed in diabetic untreated control. Hematological parameters assessment is useful in explaining the hematological functions of a chemical compound or plant extracts *in vivo*[42]. In the present study, the GON and TO leaf extracts demonstrated varying degrees of influences on hematological parameters. The extract significantly increased the levels of WBC, RBC, HGB, HCT, PLT, LYM and MON in treated rats. The observed increased in RBC, HGB and PCV levels upon administration of the leaf extract of GON and TO suggest that the extract could have stimulated enhanced activity of erythropoietin release in the kidney, a regulator of RBC production[42]. The presences of phytochemicals like flavonoids, tannins and terpenes in the GON and TO extracts may be responsible for the haemopoietic stimulating effects[38]. This is in agreement with previous studies that showed that oral administration of antioxidant supplements of plant extracts significantly increased cells of hematopoietic origin in animals, since phytochemicals can protect erythrocytes from oxidative damage[43].

On examination of the liver photomicrographs for the diabetic untreated group, they were prominent characteristics such as mentioned in the work of Mohamed *et al.*[33]. These features were observed to have been ameliorated in the treatment groups, although they were not completely reversed. Under close observations, some of the changes evident in the photomicrographs for the diabetic treated groups showed significant regeneration and reversal of toxic effects seen in the diabetes mellitus. The TEL and GON treated groups were not as efficient as the combined group in the restoration of the liver architecture with profound damages observed in the diabetic untreated groups.

Hyperglycaemia occasioned by the insufficiency of insulin or inability of the cells to utilize glucose is often accompanied with multiple of other medical complications especially when it is untreated. Medicinal plants have been reported to possess hypoglycemic principles. These plants have been used by folk medicine practitioners for diabetic treatment. This study has also confirms that the crude extracts of GON and TO in combination, produced a more potent effect in ameliorating the complications associated with diabetes mellitus when compared to the single plants. The study therefore ascertains the claim that *G. latifolium* and *T. occidentalis* are endowed with bioactive constituents that can ameliorate the complications associated with diabetes. However, detailed study of the molecular mechanism of action of these potential antidiabetics using specific molecular markers in diabetes need to be evaluated in subsequent studies to affirm the result of this preliminary study if the required resources are made available.

CONCLUSION

Dichloromethane and ethanolic leave extracts of *T. occidentalis* and *G. latifolium* possess hypoglycaemic property and hepatic tissue regeneration ability in STZ induced diabetic Wistar rats. These findings support the claim by traditional herbal healers that these leaves extracts are effective in the management of diabetes mellitus and also demonstrated that the leave extracts, in combination

(synergy), possess a more potent ability, hence a better regimen for the treatment and management of diabetes with very minimal side effect.

SIGNIFICANCE STATEMENT

The present study discovered a possible synergistic effect of *Telfeira occidentalis* and *Gongronema latifolia* solvent extracts in ameliorating complications occasioned by diabetes in Wistar rats. More so, its present a positive impact of phyto-active compounds in preserving the integrity of the red blood cells and other cells involved in immunological cascade in diabetic condition. Hence, this study will take a lead in the discovery of mechanism of action of synergism in phyto-active agents with little or no side effects from both plants.

REFERENCES

1. Sonkamble, V.V., N.S. Wagh and S.R. Pai, 2019. Role of Plant Secondary Metabolites as Antidiabetic Agents. In: Natural Bio-active Compounds, Volume 1: Production and Applications, Akhtar, M.S., M.K. Swamy and U.R. Sinniah (Eds.). Springer, Singapore, ISBN: 978-981-13-7153-0, pp: 529-550.
2. El-Abhar, H.S. and M.F. Schaalan, 2014. Phytotherapy in diabetes: Review on potential mechanistic perspectives. World J. Diabetes., 5: 176-197.
3. Patel, D.K., R. Kumar, D. Laloo and S. Hemalatha, 2012. Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. Asian Pac. J. Trop. Dis., 2: 239-250.
4. WHO., 2018. Diabetes: Key facts. World Health Organization, Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/diabetes
5. CDC., 2017. National diabetes statistic report, 2017. National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA., USA.
6. Rui, L., 2011. Energy metabolism in the liver. Compr. Physiol., 4: 177-197.
7. Hall, P. and J. Cash, 2012. What is the real function of the liver 'function' tests? Ulster Med. J., 81: 30-36.
8. Christopher, S.L., O.S.O. Ejuoghanran and O.A. Festus, 2015. Profertility effects of aqueous leaf extract of *Telfairia occidentalis* in adult male Wistar rats. J. Exp. Clin. Anat., 14: 88-94.
9. Salman, T.M., I.A. Alagbonsi and A.R.A. Feyitimi, 2018. Role of reactive oxygen species-total antioxidant capacity status in *Telfairia occidentalis* leaves-associated spermatoprotective effect: A pointer to fatty acids benefit. J. Basic Clin. Physiol. Pharmacol., 29: 347-358.
10. Idris, S., 2011. Compositional studies of *Telfairia occidentalis* leaves. Am. J. Chem., 1: 56-59.
11. Christian, A., 2007. Fluted pumpkin (*Telfairia occidentalis* Hook f.) seed: A nutritional assessments. Electron. J. Environ. Agric. Food Chem., 6: 1787-1793.
12. Akindele, A.J., M.Y. Ajao, F.R. Aigbe and U.S. Enumah, 2013. Effects of *Telfairia occidentalis* (Fluted Pumpkin; Cucurbitaceae) in mouse models of convulsion, muscle relaxation and depression. J. Med. Food, 16: 810-816.
13. Eseyin, O.A., A.C. Igboasoiyi, E. Oforah, H. Mbagwu, E. Umoh and J.F. Ekpe, 2005. Studies of the effects of alcohol extract of *Telfairia occidentalis* on alloxan induced diabetic rats. Global J. Pure Applied Sci., 11: 77-79.
14. Kayode, A.A.A., O.T. Kayode and A.A. Odetola, 2010. *Telfairia occidentalis* ameliorates oxidative brain damage in malnorished rats. Int. J. Biol. Chem., 4: 10-18.
15. Adaramoye, O.A., J. Achem, O.O. Akintayo and M.A. Fafunso, 2007. Hypolipidemic effect of *Telfairia occidentalis* (fluted pumpkin) in rats fed a cholesterol-rich diet. J. Med. Food, 10: 330-336.
16. Oboh, G. and A.A. Akindahunsi, 2004. Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutr. Health, 18: 29-36.
17. Edet, E.E., M.I. Akpanabiatu, F.E. Uboh, T.E. Edet, A.E. Eno, E.H. Itam and I.B. Umoh, 2011. *Gongronema latifolium* crude leaf extract reverses alterations in haematological indices and weight-loss in diabetic rats. J. Pharmacol. Toxicol., 6: 174-181.
18. Udoh, F.V., G.A. Eshiet, J.O. Akpan and F.E. Edu, 2013. Hypoglycemic effect of *Gongronema latifolia* extracts in rats. J. Nat. Sci. Res., 3: 37-44.
19. Eleyinmi, A.F., 2007. Chemical composition and antibacterial activity of *Gongronema latifolium*. J. Zhejiang Univ. Sci. B, 8: 352-358.
20. Atawodi, S.E., 2005. Antioxidant potential of African medicinal plants. Afr. J. Biotechnol., 4: 128-133.
21. Akinnuga, A.M., O. Bamidele, P. Ekechi and O.S. Adeniyi, 2011. Effects of an ethanolic leaf extract of *Gongronema latifolium* on haematological some parameters in rats. Afr. J. Biomed. Res., 14: 153-156.
22. Ugochukwu, N.H., N.E. Babady, M. Cobourne and S.R. Gasset, 2003. The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J. Biosci., 28: 1-5.
23. Morebise, O., M.A. Fafunso, J.M. Makinde, O.A. Olajide and E.O. Awe, 2002. Antiinflammatory property of the leaves of *Gongronema latifolium*. Phytother. Res., 16: 75-77.
24. NIH., 1985. Guide for the care and use of laboratory animals. NIH Publications No. 85-23, National Institutes of Health, USA.
25. Esmerino, L.A., R. Ranali and A.L. Rodrigues Jr., 1998. Blood glucose determination in normal and alloxan-diabetic rats after administration of local anesthetics containing vasoconstrictors. Braz. Dent. J., 9: 33-37.

26. Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.
27. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
28. Tietz, N.W., 1995. Clinical Guide to Laboratory Tests. 3rd Edn., W.B. Saunders, Philadelphia, USA., ISBN-13: 9780721650357, pp: 518-519.
29. Szasz, G., 1976. Reaction-rate method for γ-glutamyltransferase activity in serum. Clin. Chem., 22: 2051-2055.
30. Jarrar, B.M. and N.T. Taib, 2012. Histological and histochemical alterations in the liver induced by lead chronic toxicity. Saudi J. Biol. Sci., 19: 203-210.
31. Shrestha, J.T.M., H. Shrestha, M. Prajapati, A. Karkee and A. Maharjan, 2017. Adverse effects of oral hypoglycemic agents and adherence to them among patients with type 2 diabetes mellitus in Nepal. J. Lumbini Med. Coll., 5: 34-40.
32. Kasole, R., H.D. Martin and J. Kimiywe, 2019. Traditional medicine and its role in the management of diabetes mellitus: "Patients' and herbalists' perspectives". Evidence-Based Complement. Altern. Med., Vol. 2019. 10.1155/2019/2835691.
33. Mohamed, J., A.H.N. Nafizah, A.H. Zariyantey and S.B. Budin, 2016. Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation. Sultan Qaboos Univ. Med. J., 16: 132-141.
34. Wang-Fischer, Y. and T. Garyantes, 2018. Improving the reliability and utility of streptozotocin-induced rat diabetic model. J. Diabetes Res., Vol. 2018. 10.1155/2018/8054073.
35. Okonwu, K., L.A. Akonye and S.I. Mensah, 2017. Phytochemical profile of *Telfairia occidentalis* leaf grown in soilless and soil media using HPLC. J. Agric. Stud., 5: 179-198.
36. El Barky, A.R., S.A. Hussein, A.E. Alm-Eldeen, Y.A. Hafez and T.M. Mohamed, 2017. Saponins and their potential role in diabetes mellitus. Diabetes Manage., 7: 148-158.
37. Mahdiani, A., M. Kheirandish and S. Bonakdaran, 2019. Correlation between white blood cell count and insulin resistance in type 2 diabetes. Curr. Diabetes Rev., 15: 62-66.
38. Sharma, K., A.K. Patel, K.H. Shah and A. Konat, 2017. Is neutrophil-to-lymphocyte ratio a predictor of coronary artery disease in Western Indians? Int. J. Inflamm., Vol. 2017. 10.1155/2017/4136126.
39. Chawla, A., R. Chawla and S. Jaggi, 2016. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian J. Endocrinol. Metab., 20: 546-551.
40. Del Rio, M.M., M. Tiwari, L.I. Amodu, J. Cagliani and H.L.R. Rilo, 2016. Glycated hemoglobin, plasma glucose and erythrocyte aging. J. Diabetes Sci. Technol., 10: 1303-1307.
41. Mockesch, B., P. Connes, K. Charlot, S. Skinner and M.D. Hardy-Dessources *et al*., 2017. Association between oxidative stress and vascular reactivity in children with sickle cell anaemia and sickle haemoglobin C disease. Br. J. Haematol., 178: 468-475.
42. Jorum, O.H., N.M. Piero and A.K. Machocho, 2016. Haematological effects of dichloromethane-methanolic leaf extracts of *carissa edulis* (Forssk.) Vahl in normal rat models. J. Hematol. Thromboembolic Dis., Vol. 4. 10.4172/2329-8790.1000232.
43. Ikumawoyi, V.O., O. Awodele, K. Rotimi and A.Y. Fashina, 2016. Evaluation of the effects of the hydro-ethanolic root extract of *Zanthoxylum zanthoxyloides* on hematological parameters and oxidative stress in cyclophosphamide treated rats. Afr. J. Tradit. Complement. Altern. Med., 13: 153-159.