Original Research Article

Comparative Effects of *Aloe vera* Gel and Aqueous Leaf Extract of *Viscum album* on Bilirubin Excretion in Streptozotocin - Induced Diabetic Rats

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ABSTRACT

Aims: This study was carried out to determine the effect of type 1 Diabetes Mellitus on bilirubin excretion, and to compare the effects of separate administration of *Aloe vera gel* and aqueous leaf extract of *viscum album* on serum bilirubin, bile secretory rate and biliary bilirubin concentration.

Methodology: Thirty six male albino wistar rats weighing 180 - 220 g were used for this study. After 14 days of habituation, the rats were randomly divided into 6 groups of 6 rats each. Type 1 Diabetes Mellitus was induced in the test groups by a single i.p dose (65 mg/kg) of streptozotocin. Group 1 served as control; group 2 - diabetic untreated group (DM); group 3 - diabetic group, treated with 0.4ml/100g *Aloe vera gel* orally (DM+Aloe); group 4 - diabetic group, treated with 150 mg/kg *Viscum album* leaf extract orally (DM+VA); group 5 - control group, treated with 0.4ml/100g *Aloe vera* gel orally (C+Aloe); group 6 - control group, treated with 150 mg/kg *Viscum album* leaf extract orally (C+VA). All animals had unrestricted access to food and water. The regimen lasted for 21 days, after which bile secretion was determined and same was collected together with serum for biliary and serum bilirubin estimation.

Results: The results showed that serum and biliary total, conjugated and unconjugated bilirubin concentrations were significantly ($p<0.001$) higher in the DM group compared to control, DM+Aloe and DM+VA, with DM+Aloe group having significantly lower serum and biliary total and conjugated bilirubin ($p<0.001$), and serum unconjugated bilirubin ($p<0.05$) compared to DM+VA group. Serum conjugated bilirubin concentration in C+Aloe and C+VA group was significantly ($p<0.05$ and $p<0.001$ respectively) higher compared to control, while serum unconjugated bilirubin concentration was significantly ($p<0.001$ and $p<0.01$ respectively) lower compared to control. C+VA group had a significant ($p<0.001$) increase in biliary total and conjugated bilirubin concentrations compared to C+Aloe group.

Conclusion: On the basis of the results obtained, we therefore conclude that *Aloe vera* gel and aqueous leaf extract of *Viscum album* enhances bilirubin excretion in diabetic and normal animals and are both hepatoprotective.

Keywords: Aloe vera, bile secretion, biliary bilirubin, serum bilirubin, Viscum album

1. INTRODUCTION

Bilirubin is a product of haemoglobin metabolism and serves as a marker for liver and blood disorders. After about 120 days, the membranes of erythrocytes become increasingly fragile and susceptible to damage [1]. As these cells attempt to squeeze through the capillaries of the reticuloendothelial system, their membranes become ruptured, and hemoglobin is released in the process [2]. The haemoglobin released in the process enters a series of reaction that leads to the formation of bilirubin. About 80 % of the daily bilirubin production is

derived from haemoglobin, while the other 20 % is derived from the breakdown of myoglobin, catalase, peroxidase, cytochromes and tryptophan pyrrolase [2,3,4,5]. Conditions associated with liver damage, increased levels of immature red blood cells in circulation or polycythemia may result in increased formation of bilirubin [3,4].

After synthesis, bilirubin reversibly binds to albumin and is transported to the liver, where it is conjugated and excreted as bile pigment. However, not all the bilirubin molecules are conjugated by the liver. The unconjugated fraction forms unconjugated bilirubin. Intestinal bacteria degrades bilirubin into urobilinogen, most of which is absorbed from the intestine and undergoes enterohepatic recirculation [6].

Although bilirubin is toxic to the body, some studies have claimed that mild increase in serum bilirubin concentration may be beneficial in treatment of some form of cancer and gastric ulcer by virtue of its antioxidant effect [5,7]. Amidst these acclaimed benefits of mild hyperbilirubinemia, several detrimental effects exist. Bilirubin is toxic to the central nervous system and may cause a sequence of neurological symptoms as observed in acute bilirubin encephalopathy [8]. Although hyperbilirubinemia is most frequently observed in infants, as seen in jaundice and kernicterus, it is becoming increasingly evident in adults, with the likely causes being, but not limited to hemolysis, liver damage and Gilbert syndrome [8,9].

Aloe is a cactus-like perennial plant belonging to family Liliaceae with over 360 species [10]. Of the about 360 species of *Aloe vera*, *Aloe vera* barbadensis has been named the species that is most effective therapeutically [11]. The *Aloe vera* plant can be utilized in three (3) basic forms; Aloe gel which is derived from the inner part of the leaves by cutting open the leaves, Aloe latex which is yellowish and derived from the leaves and the whole leaf extract which is obtained by blending the entire leaf. The latex of *Aloe vera* contains the anthraquinone glycosides aloin A and B, which are potent laxatives [12,13].

Viscum album, belonging to family Loranthaceae, is an evergreen semi-parasitic plant that grows primarily on the branches of deciduous trees. The plant is widely distributed in Australia, Europe, Asia, North Africa and also in Nigeria. Leaf extract of *Viscum album* has been reported as being useful in the treatment of diabetes mellitus, cholera, cancer, epilepsy, wounds, tumor, asthma, anxiety, amenorrhea, atherosclerosis and headache associated with hypertension [14,15,16].

Since diabetes is a metabolic disorder, the possibility of altered or impaired liver functions in diabetics cannot be overemphasized. Both *Aloe vera* gel and *Viscum album* have been reported to be beneficial in the treatment of diabetes mellitus (DM) [10,17,18]. Our study was aimed at determining the effect of type one diabetes mellitus on bilirubin excretion, by measuring serum and biliary bilirubin concentrations as well as bile secretory rate, and to compare the impact of treatment with either *Aloe vera* gel or *Viscum album* on same.

2. MATERIAL AND METHODS

2.1 Plant Material and Preparation of Leaf Extracts

Mature, fresh *Aloe vera* plant with leaves within 40 – 70 cm long were obtained from University of Calabar Botanical Garden. The leaves were rinsed with clean water to remove debris and sand, and thereafter mopped with a dry cloth. They were then sliced longitudinally to expose the *Aloe vera* gel. The gel was gently scraped into an electric blender to shatter the block. This preparation was done daily and administered to the animals without storage.

Fresh leaves of *Viscum album* (mistletoe) were collected from a host plant (citrus) in Calabar South local government area of Cross River state, Nigeria. The leaves were rinsed with clean water to remove debris and sand. They were first air dried, and subsequently transferred into the AstellHearson oven where they were dried at 40 - 45^{O}C. The dried leaves were ground to powder using an electric blender to obtain 1500 g. The dry sample was peculated in 7.5 L distil water for 24 hours. The mixture was then filtered with size 1 Whatman's filter paper. The filtrate was oven dried at 45^{O}C. The pasty filtrate obtained after drying was weighed using a mettler P163 electronic weighing balance. 1500 mg/ml concentration of the stock solution of the extract was obtained by dissolving 15 g of extract in 10ml of distil water. The stock solution was labeled appropriately and refrigerated at 4^{O}C until required for use.

Both plant materials were identified by the chief Herbarium officer of Botany department of the University of Calabar, Cross River State, Nigeria. The median lethal dose (LD_{50}) of the plant extracts were determined by method of Lorke (1983) [19].

2.2 Animal Preparation and Protocol

Thirty six male albino wistar rats weighing 180 - 220 g were used for this study. The animal cages were well ventilated, exposed to normal temperature and 12/12 hours light/dark cycle. After fourteen days of habituation, the animals were randomly assigned one of six groups such that each group contained 6 animals. The groups were labeled as follows; group 1 - control; group 2 - streptozotocin – induced diabetic untreated group (DM); group 3 - streptozotocin – induced diabetic group, treated with *Aloe vera* gel (DM+Aloe); group 4 - streptozotocin - induced diabetic group, treated with leaf extract of *Viscum album* (DM+VA); group 5 - control group, treated with *Aloe vera* gel (C+Aloe) and group 6 - control group, treated with leaf extract of *Viscum album*(C+VA). All animals had unrestricted access to food and water.

2.2.1 Induction of diabetes

Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) at a one time dose of 65 mg/kg. Fasting blood glucose level of each animal was taken before STZ administration. 48 hours after STZ administration, diabetes was confirmed in the groups administered by using the Finetest glucose meter (IMFOMED IMPEX, INDIA) to measure the blood glucose levels. Animals with blood glucose level >200 mg/dl after 24 hours fast were selected for this study.

2.2.2 Extract administration

Aloe vera gel was administered to the DM+Aloe and C+Aloe groups at a dose of 0.4ml/100g body weight, while aqueous leaf extract of *Viscum album* was administered to the DM+VA and C+VA groups at a dose of 150 mg/kg body weight. The extracts were administered orally, once daily for 3 weeks. Administration was facilitated by the use of a syringe and orogastric tube. All experiments involving the animals and their care were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.3 Determination of Blood Glucose Levels

Fasting blood glucose level of the animals was measured using the Finetest glucose meter (INFOMED IMPEX, INDIA). Blood used for the test was obtained by pricking the distal end of the tail and placing the drop of blood on the test strip. Fasting blood glucose level before and

after STZ administration were determined and recorded in all the groups. Fasting blood glucose level was also measured before sacrifice.

2.4 Determination of Serum and Biliary Bilirubin Concentration

Serum bilirubin concentration was measured by the method described by Sherlock [20], while biliary bilirubin concentration was measured by colorimetric method as described by Jendrassik and Grof, [21].

2.5 Determination of Rate of Biliary Secretion

Biliary secretion was collected by the method of Vickers *et al* [22]. After 12 hours fast, the animals were weighed and anaesthesized by intraperitoneal administration of sodium thiopentone (6mg/100g body weight), and were quickly pinned to a dissecting board for a tracheostomy performed to ease breathing. The stomach was then opened along the linea alba to minimize bleeding. A laparotomy was performed and the liver lobes deflected anterolaterally to expose the common bile duct. Using a portex Cannula (0.5mm in diameter), the common bile duct was cannulated after a small incision was made. A thread was used to tie round the common bile duct to hold the cannula in place. The bile content was collected at 3 hours interval.

2.6 Determination of Percentage Serum and Biliary Conjugated Bilirubin Concentration

Percentage serum conjugated bilirubin (SCB) concentration was determined mathematically, using the formula:

$$\text{Percentage SCB} = \frac{\text{Serum conjugated bilirubin concentration}}{\text{Serum total bilirubin concentration}} \times 100$$

Percentage biliary conjugated bilirubin (BCB) concentration was determined mathematically, using the formula:

$$\text{Percentage BCB} = \frac{\text{Biliary conjugated bilirubin concentration}}{\text{Biliary total bilirubin concentration}} \times 100$$

The results were recorded and differences analyzed statistically.

2.7 Statistical Analysis

Results are presented as mean $\pm$ standard error of mean. The One – way Analysis of Variance (ANOVA) was used to determine the differences between means, followed by post hoc multiple comparisons. P=.05 was considered significant. Computer software SPSS version 17.0 and Microsoft Excel (2007 version) Analyzer were used for the analysis.

3. RESULTS AND DISCUSSION

3.1 Fasting Blood Glucose Concentration in the Different Experimental Groups

There was no significant difference in the fasting blood glucose concentration of animals in the different experimental groups before STZ administration (Fig. 1). Forty eight hours after STZ administration, the mean fasting blood glucose concentration in the different experimental groups was 67 ± 2.5, 226 ± 5.2, 228 ± 3.0, 227 ± 3.7, 68 ± 2.9 and 67 ± 2.0 mg/dl for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. Mean fasting blood glucose level was significantly ($p<0.001$) higher in DM, DM+Aloe and DM+VA groups compared to control (Fig. 1). Before sacrifice, mean fasting blood glucose in the different experimental groups were 70 ± 1.9, 226 ± 2.7, 70 ± 1.8, 87 ± 1.7, 72 ± 1.3 and 74 ± 1.7 mg/dl for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. Mean fasting blood glucose concentration before sacrifice was significantly ($p<0.001$) reduced in DM+Aloe and DM+VA groups, compared to DM group, with DM+Aloe being significantly ($p<0.001$) lower, compared to DM+VA group, (Fig. 1).

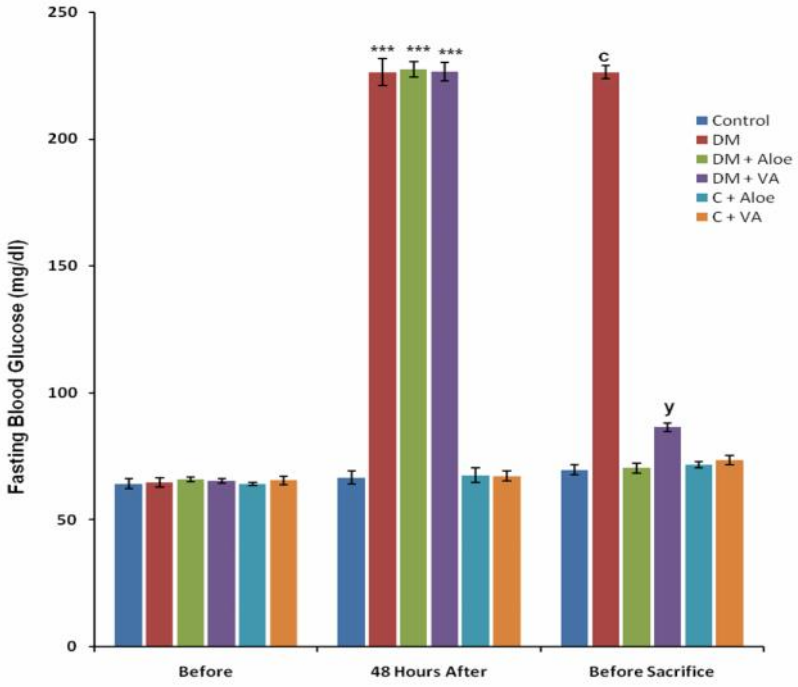

Fig. 1. Comparison of fasting blood glucose level in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001 vs control, C+Aloe, C+VA; c = p<0.001 vs control, DM+Aloe, DM+VA; y = p<0.001 vs DM+Aloe.

3.2 Serum Total, Conjugated and Unconjugated Bilirubin Concentrations

3.2.1 Serum total bilirubin concentration

Serum total bilirubin (STB) concentration in the different experimental groups was 4.3 ± 0.14, 9.0 ± 0.25, 3.7 ± 0.15, 5.4 ± 0.12, 3.6 ± 0.12 and 3.9 ± 0.08 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. STB concentration was significantly ($p<0.001$) increased in DM group, compared to control. DM+Aloe and DM+VA group had a significantly ($p<0.001$) lower STB concentration compared to DM group, with DM+Aloe group having a significantly ($p<0.001$) lower STB concentration, compared to DM+VA.

C+Aloe group had a significantly ($p<0.05$) lower STB concentration, compared to control while that of C+VA group was not significantly different from control, (Fig. 2).

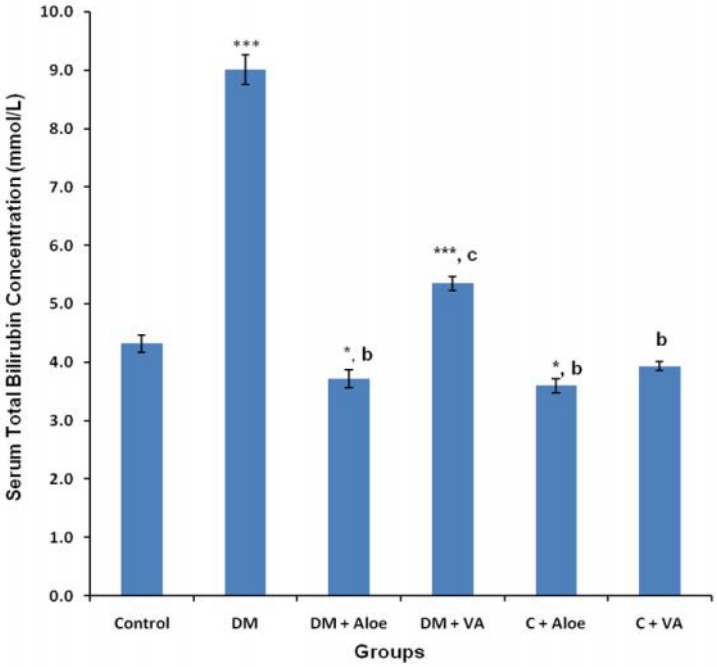

Fig. 2. Comparison of serum total bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001, *p<0.05 vs control; b = p<0.001 vs DM, DM+VA; c = p<0.001 vs DM.

3.2.2 Serum conjugated bilirubin concentration

Serum conjugated bilirubin (SCB) concentration in the different experimental groups was 2.5 ± 0.08, 3.5 ± 0.13, 2.4 ± 0.07, 3.5 ± 0.10, 2.9 ± 0.12 and 3.0 ± 0.07 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. SCB concentration was significantly ($p<0.001$) increased in DM and DM+VA groups, compared to control. DM+Aloe group had a significantly ($p<0.001$) lower SCB concentration compared to DM and DM+VA groups. SCB concentration was significantly increased in C+Aloe group ($p<0.05$) and C+VA group ($p<0.01$), compared to control. SCB concentration in the C+Aloe group was not significantly different compared to that of C+VA group, (Fig. 3).

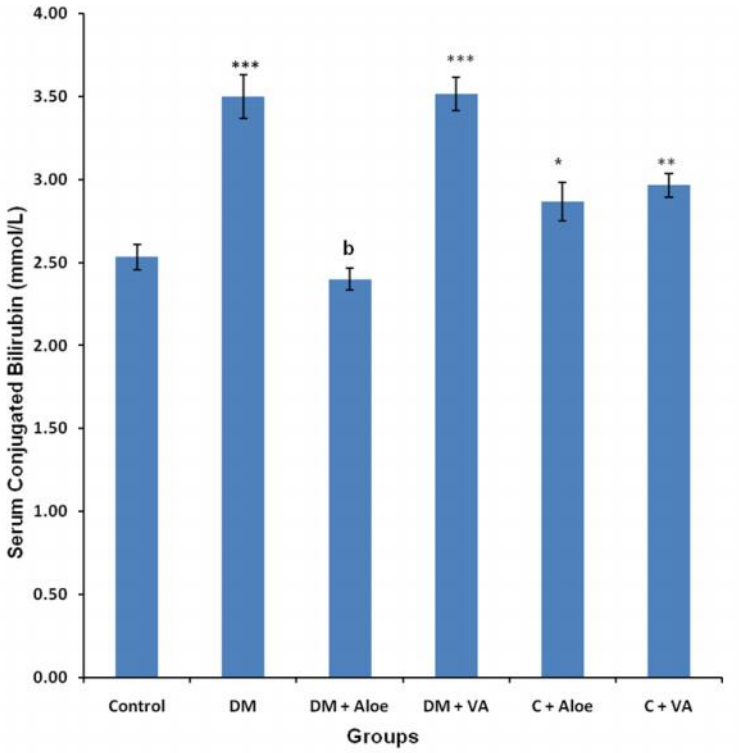

Fig. 3. Comparison of serum conjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

*p<0.05, **p<0.01, ***P<0.001 vs control; b = p<0.001 vs DM, DM+VA.

3.2.3 Serum unconjugated bilirubin concentration

Serum unconjugated bilirubin (SUB) concentration in the different experimental groups was 1.8 ± 0.14, 5.5 ± 0.28, 1.3 ± 0.19, 1.8 ± 0.10, 0.7 ± 0.14 and 1.0 ± 0.03 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. SUB concentration was significantly ($p<0.001$) increased in DM group, compared to control. SUB concentration was significantly ($p<0.001$) reduced in DM+Aloe and DM+VA groups, compared to DM group, with DM+Aloe group being significantly ($p<0.05$) lower compared to DM+VA group. SUB concentration was significantly reduced in C+Aloe group ($p<0.001$) and C+VA group ($p<0.01$), compared to control. SUB concentration in the C+Aloe group was not significantly different compared to C+VA group, (Fig. 4).

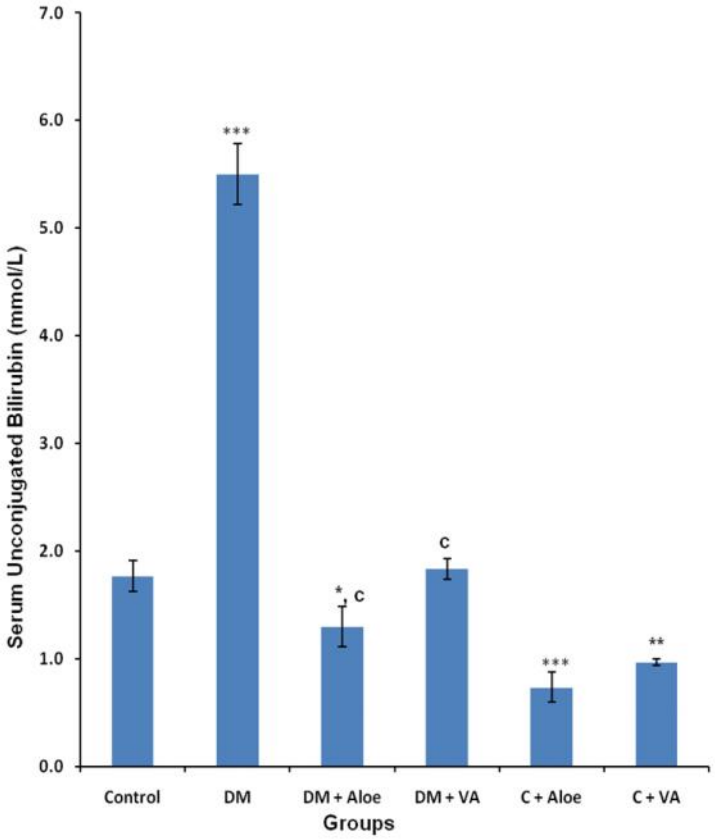

Fig. 4. Comparison of serum unconjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

**p<0.01, ***p<0.001 vs control, c = p<0.001 vs DM; *p<0.05 vs DM+VA.

3.3 Biliary Total, Conjugated and Unconjugated Bilirubin Concentrations

3.3.1 Biliary total bilirubin concentration

The mean biliary total bilirubin (BTB) concentration in the different experimental groups was 6.4 ± 0.14, 16.8 ± 0.42, 4.4 ± 0.11, 7.3 ± 0.32, 4.4 ± 0.22 and 6.9 ± 0.15 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. BTB concentration was significantly ($p<0.001$) increased in DM group, compared to control. DM+Aloe and DM+VA groups had a significantly ($p<0.001$) lower BTB concentration compared to DM group, with DM+Aloe group having a significantly ($p<0.001$) lower BTB concentration, compared to DM+VA.

C+Aloe group had a significantly ($p<0.05$) lower BTB concentration, compared to control while that of C+VA group was not significantly different from control, (Fig. 5).

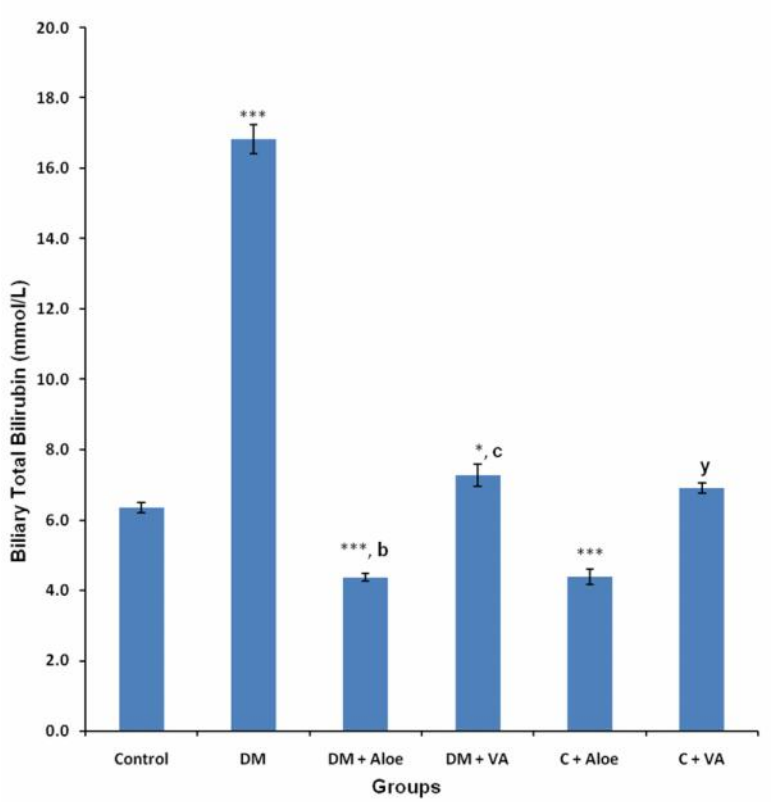

Fig. 5. Comparison of biliary total bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

*p<0.05, ***p<0.001 vs control; b = p<0.001 vs DM, DM+VA; c = p<0.001 vs DM; y = p<0.001 vs C+Aloe.

3.3.2 Biliary conjugated bilirubin concentration

The mean biliary conjugated bilirubin (BCB) concentration in the different experimental groups was 4.8 ± 0.31, 10.1 ± 0.27, 3.4 ± 0.15, 5.5 ± 0.29, 3.4 ± 0.21 and 5.6 ± 0.15 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. BCB concentration was significantly (p<0.001) increased in DM group, compared to control. DM+Aloe and DM+VA groups had a significantly (p<0.001) lower BCB concentration compared to DM group, with DM+Aloe group having a significantly (p<0.001) lower BCB concentration, compared to DM+VA.

C+Aloe group had a significantly (p<0.001) lower BCB concentration, compared to control while C+VA group had a significantly (p<0.05) higher BCB concentration compared to control. BCB concentration was significantly (p<0.001) reduced in C+Aloe group compared to C+VA group, (Fig. 6).

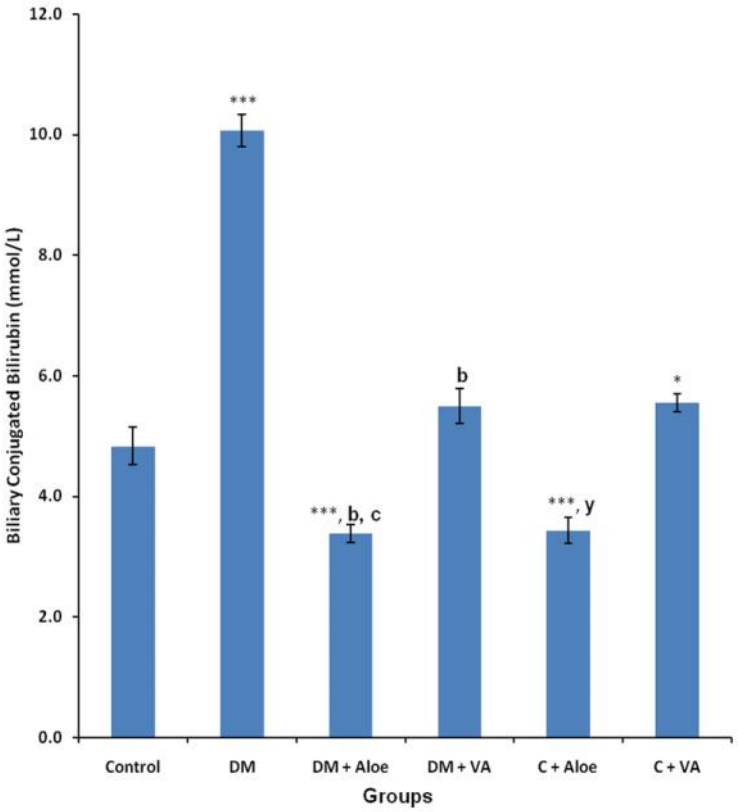

Fig. 6. Comparison to biliary conjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001, *p<0.05 vs control; b = p<0.001 vs DM; c = p<0.001 vs DM+VA; y = p <0.001 vs C+VA.

3.3.3 Biliary unconjugated bilirubin concentration

The mean biliary unconjugated bilirubin (BUB) concentration in the different experimental groups was 1.5 ± 0.39, 6.8 ± 0.52, 1.0 ± 0.12, 1.8 ± 0.14, 1.0 ± 0.10 and 1.4 ± 0.15 mmol/L for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. BUB concentration was significantly ($p<0.001$) increased in DM group, compared to control. DM+Aloe and DM+VA groups had a significantly ($p<0.001$) lower BUB concentration compared to DM group. BUB concentration in DM+Aloe group was not significantly different compared to DM+VA.

C+Aloe and C+VA groups had a significantly ($p<0.001$) lower BUB concentration, compared to DM group. BUB concentration in C+Aloe and C+VA groups was not significantly different compared to control, (Fig. 7).

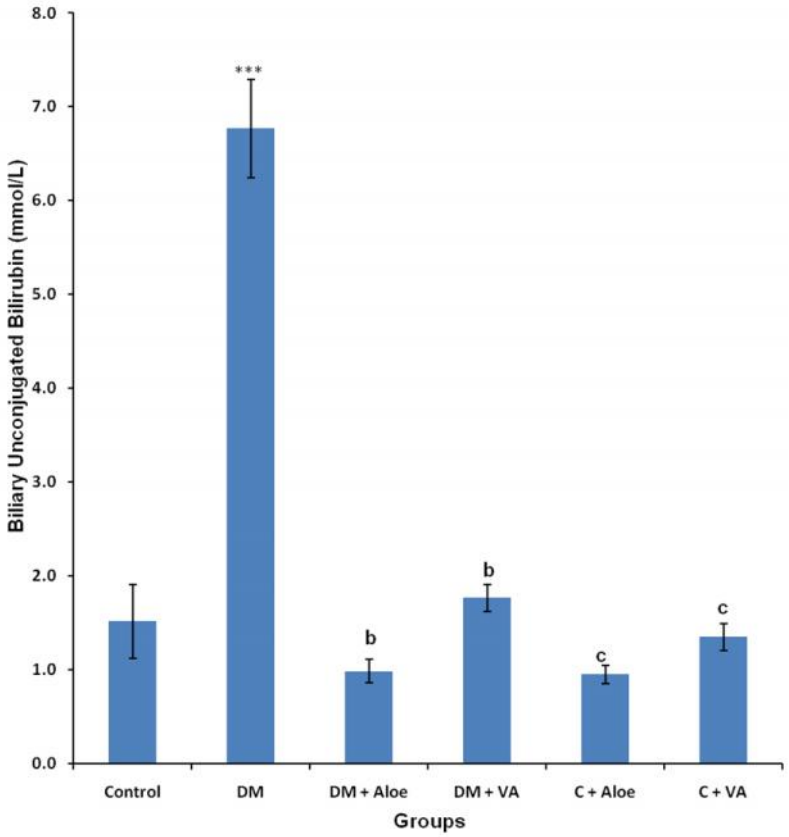

Fig. 7. Comparison of biliary unconjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001 vs control; b = p<0.001 vs DM; c = p<0.001 vs DM.

3.4 Rate of Bile Secretion

The mean bile secretory rate in the different experimental groups was 0.33 ± 0.05, 0.38 ± 0.04, 0.37 ± 0.05, 0.38 ± 0.08, 0.35 ± 0.05 and 0.40 ± 0.09 ml/hr for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. There was no significant difference in the mean rate of bile secretion in the groups study, (Fig. 8).

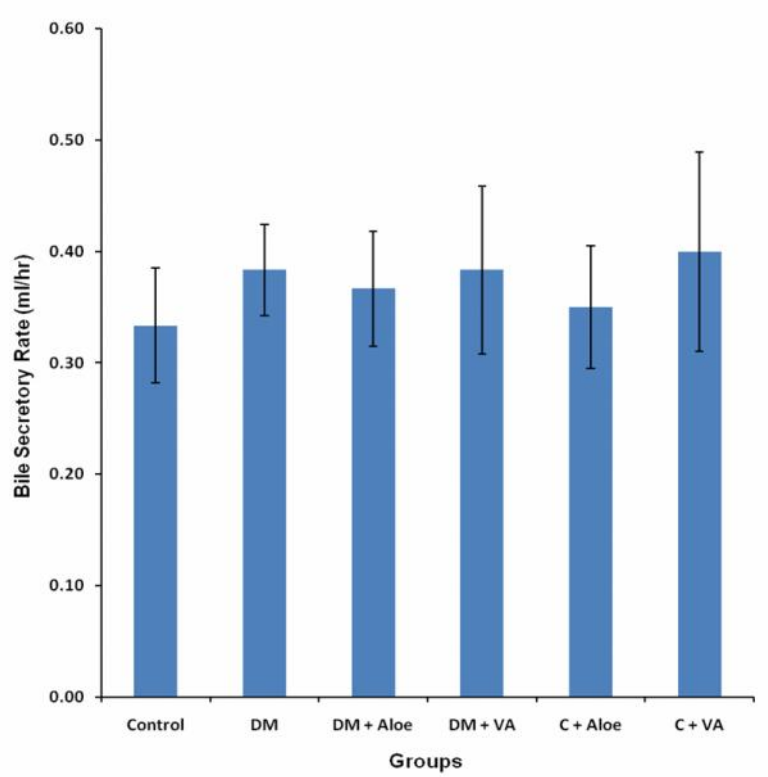

Fig. 8. Comparison of mean bile secretory rate in the different experimental groups. Values are mean ± SEM, n = 6.

3.5 Percentage Serum Conjugated Bilirubin Concentration

The percentage serum conjugated bilirubin (SCB) concentration in the different experimental groups was 58.9 ± 2.2, 38.9 ± 1.7, 64.85 ± 4.3, 65.76 ± 1.5, 79.9 ± 3.7 and 75.41 ± 0.8 % for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. Percentage SCB concentration was significantly ($p<0.001$) reduced in DM group, compared to control. DM+Aloe and DM+VA groups had a significantly ($p<0.001$) higher percentage SCB concentration compared to DM group. C+Aloe group had a significantly ($p<0.001$) higher percentage SCB concentration, compared to control, (Fig. 9).

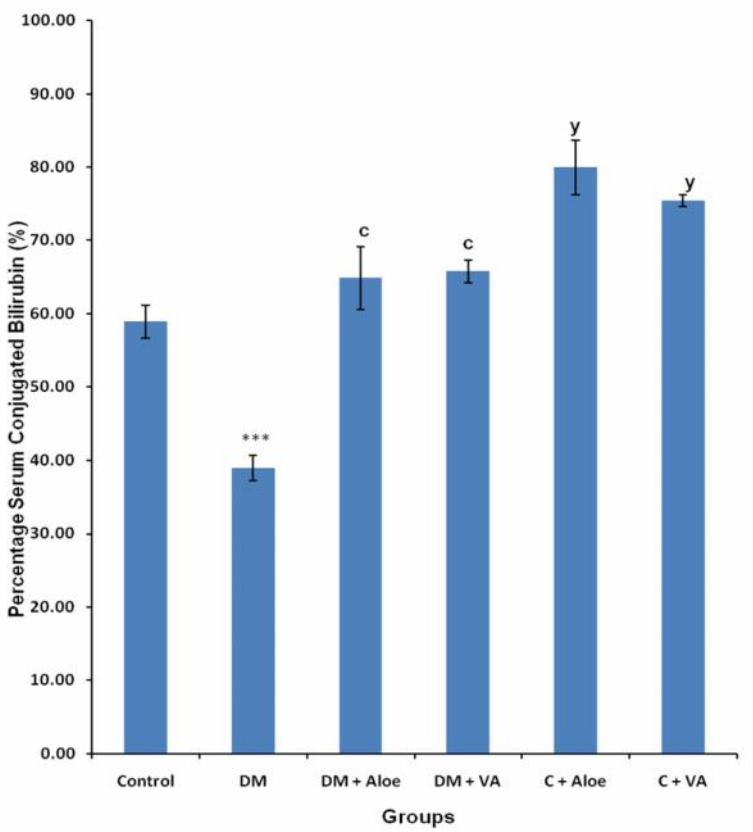

Fig. 9. Comparison of the percentage serum conjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001, y = p<0.001 vs control; c = p<0.001 vs DM

3.6 Percentage Biliary Conjugated Bilirubin Concentration

The percentage biliary conjugated bilirubin (BCB) concentration in the different experimental groups was 76.5 ± 5.6, 60.0 ± 2.3, 77.5 ± 2.9, 75.7 ± 1.8, 78.2 ± 1.9 and 80.5 ± 1.9 % for control, DM, DM+Aloe, DM+VA, C+Aloe and C+VA group respectively. Percentage BCB concentration was significantly ($p<0.001$) reduced in DM group, compared to control. Percentage BCB concentration was significantly increased in DM+Aloe ($p<0.001$) and DM+VA ($p<0.01$) groups, compared to DM group. C+Aloe and C+VA groups had a significantly ($p<0.001$) higher percentage BCB concentration, compared to DM group, (Fig. 10).

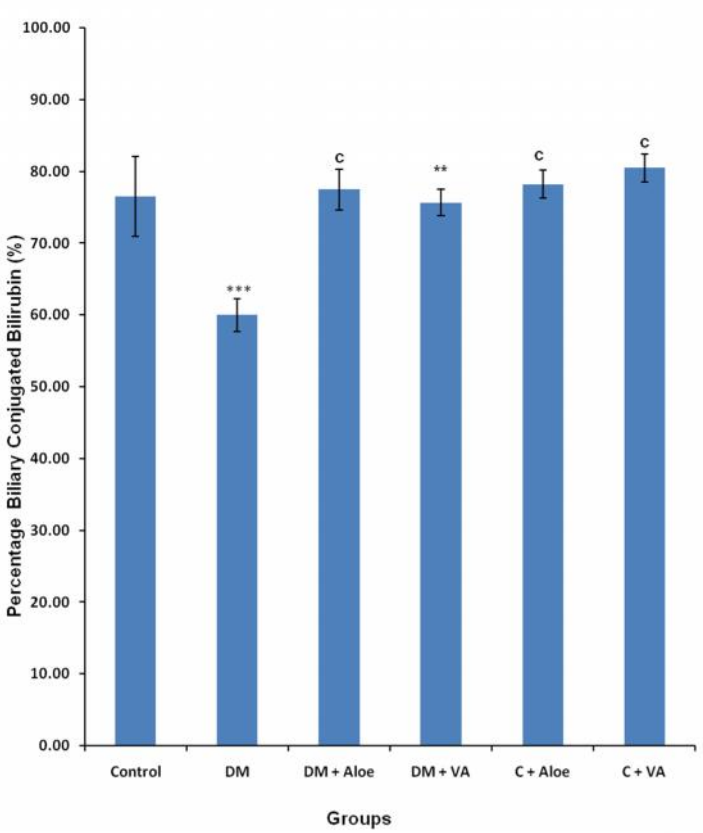

Fig. 10. Comparison of percentage biliary conjugated bilirubin concentration in the different experimental groups. Values are mean ± SEM, n = 6.

***p<0.001 vs control; **p<0.01, c = p<0.001 vs DM.

4. DISCUSSION

Diabetes mellitus is known to be associated with chronic hyperglycemia. Determination of fasting blood glucose levels 48 hours after STZ administration, confirmed type 1 diabetes mellitus (T1DM) in the groups administered, suggesting that the insulin producing pancreatic beta cells were destroyed by streptozotocin (Fig. 1). Nna *et al* [10] and Obatomi *et al* [17] had earlier reported the hypoglycemic property of *Aloe vera* gel and *Viscum album* respectively. This present study is consistent with previous reports [10,17] that *Aloe vera* gel and *Viscum album* leaf extract ameliorate derangements in blood glucose levels in T1DM.

Estimation of serum bilirubin is often instrumental in determining the state of health of the liver, since it plays a central role in bilirubin excretion. Increased serum bilirubin concentration may result from liver damage, presence of immature red blood cells in circulation or Gilbert syndrome.

Serum and biliary total, conjugated and unconjugated bilirubin concentrations were raised in diabetic animals in our study, (Fig. 2 - 7). *Aloe vera* gel significantly reduced serum total, conjugated and unconjugated bilirubin concentrations in the diabetic group in a pattern which was significantly different from that of *Viscum album* leaf extract. The decrease in serum bilirubin concentration was more in the *Aloe vera* treated group, compared to the *Viscum album* treated group. Although serum bilirubin concentrations were reduced in *Aloe vera*

treated control animals, the decrease was not significant compared to *Viscum album* treated control animals. The decrease in serum and biliary bilirubin concentrations observed in the groups administered *Aloe vera* gel or *Viscum album* was not directly linked to the rate of bile secretion. This is evident in figure 8, which showed no significant differences in bile secretory rate in the groups studied. The decrease in serum and biliary bilirubin observed in extract treated groups in this study points to efficient conjugation and excretion by the liver through the small intestine, rather than rate of bile secretion.

El-Serag and Everhart [23], and Liane *et al* [24] both reported liver damage in diabetes mellitus. This may be responsible for the observed significant reduction in percentage serum and biliary conjugated bilirubin concentration in DM group, (Fig. 9 and 10). *Aloe vera* gel and *Viscum album* reversed the reduction in percentage serum and biliary conjugated bilirubin concentrations observed in the DM group. This feature may be attributed to their phytoconstituents, which have been proven to be hepotoprotective [25,26]. Phytochemical analysis of extracts from leaf of viscum album have shown that it contains phenylpropan, flavonoid derivatives, phenolic compounds, n-butanolic fractions, tanins, saponins, lectin, viscotoxins, arabinogalactans and choline - a derivative of acetylcholine; while *Aloe vera* contains some enzymes like alkaline phosphatase, amylase, carboxypeptidase, catalase, cellulase, lipase and peroxidase [27,28].

Reports on antioxidant effects of hyperbilirubinemia are yet unclear. Some studies have reported beneficial effects of hyperbilirubinemia in reducing the risk of cardiovascular diseases owing to its antioxidant effect [29,30], yet some have reported that there are no correlations between hyperbilirubinemia and reduced risk of cardiovascular diseases [31]. Contrary to published findings in the cardiovascular literature which suggest that bilirubin Improves vascular reactivity and cardiovascular risk in people without diabetes [32,33,34], Susie *et al* [31] reported that total bilirubin did not seem to have any beneficial effect on vascular reactivity in individuals with diabetes despite the fact that bilirubin levels were higher in diabetic group.

It is on the basis of the above inconsistencies that we suggest that relying on the antioxidant effects of *Aloe vera* gel and *Viscum album* [35,36,37,38] in the course of treating hyperbilirubinemia in diabetics remain safer, as hyperbilirubinemia may be detrimental to health.

5. CONCLUSION

On the basis of the results obtained from this study, we therefore conclude that *Aloe vera* gel and aqueous leaf extract of *Viscum album* are effective in ameliorating altered bilirubin indices triggered by T1DM. Both plant materials also reduce serum bilirubin concentrations in normal animals by increasing the rate of bilirubin conjugation in the liver, and hence influence the rate of excretion.

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CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lathe GH. The degradation of haem by mammals and its excretion as conjugated bilirubin. *Essays Biochem*. 1972;8:107-48.
2. Muraca M, Fevery J, Blanckaert N. Analytic aspects and clinical interpretation of serum bilirubins. *Semin Liver Dis*. May 1988;8(2):137-147.
3. Westwood A. The analysis of bilirubin in serum. *Ann Clin Biochem*. Mar 1991;28 (pt 2):119-130.
4. Iyanagi T, Emi Y, Ikushiro S. Biochemical and molecular aspects of genetic disorders of bilirubin metabolism. *Biochim Biophys Acta*. Sep 30 1998;1407(3):173-184.
5. Drummond GS, Valaes T, Kappas A. Control of bilirubin production by synthetic heme analogs: pharmacologic and toxicologic considerations. J Perinatol 1996; 16:S72.
6. Watson CJ. The urobilinoids: milestones in their history and some recent developments. In: Berk PD, Berlin NI, editors. Bile pigments: chemistry and physiology. Washington, DC: US Government Printing Office; 1977. p. 469–482.
7. Cuadrado A, Rojo AI. Heme oxygenase-1 as a therapeutic target in neurodegenerative diseases and brain infections. *Curr Pharm Des.* 2008;14(5):429–442.
8. Volpe JJ. Bilirubin and Brain Injury. In *Neurology of the Newborn*. 2003;521-546.
9. Muraca M, Fevery J, Blanckaert N. Relationships between serum bilirubins and production and conjugation of bilirubin. Studies in Gilbert's syndrome, Crigler-Najjar disease, hemolytic disorders, and rat models. *Gastroenterology*. 1987;92(2):309-317.
10. Nna VU, Paul UA, Ofem EO, Osim EE. Crude *Aloe vera* gel reverses polyphagia, polydipsia, hyperglycaemia and increases body weight in alloxan - induced diabetic rats. *British Journal of Medicine and Medical Research*. 2013;3(4):2306-2316.
11. Gong M, Wang F, Chen Y. Study on application of arbuscular-mycorrhizas in growing seedings of *Aloe vera* (in Chinese). Zhong yao cai = Zhongyaocai = Journal of Chinese Medicinal Materials. 2002;25(1):1–3.
12. Barcroft A. *Aloe vera:* Nature's Silent Healer. (www.jojaffa.com, http://www.aloeverahealer.com). 1999;Chapt 4.
13. Heggers JP, Elzaim H, Garfield R. Effect of the combination of Aloe vera, nitroglycerin, and L-NAME on wound healing in the rat excisional model. Journal of Alternative and Complementary Medicine. 1997:3(2):149–53.

14. Tyler VE. The honest herbal: a sensible guide to the use of herbs and related remedies. New York: Pharmaceutical Products Press, 1992:xviii, 375.
15. Lenartz D, Andermahr J, Plum G, Menzel J, Beuth J. Efficiency of treatment with galactoside-specific lectin from mistletoe against rat glioma. Anticancer Research 1998;18:1011-1014.
16. Zarkovic N, Zarkovic K, Grainca S, Kissel D, Jurin M. The Viscum album preparation Isorel inhibits the growth of melanoma B16F10 by influencing the tumor-host relationship. Anticancer Drugs 1997; 8:S17-S22.
17. Obatomi DK, Bikomo EO, Temple VJ. Antidiabetic properties of African mistltoe in streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 1994;43:13-17.
18. Oluwatosin A, Massoud A, Mehran H, Parvin P, Ali M. Methanolic extract of African mistletoe (Viscum album) improves carbohydrate metabolism and hyperlipidemia in streptozotocin-induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*. 2012;427-433. Doi: 10.1016/S1995-7645(12)60073-X.
19. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983;54:275-287.
20. Sherlock S, Lunec J. Free radical and antioxidant system in health and disease. British Journal of Hospital Medicine. 1951;43:334-344.
21. Jendrassik L, Grof P. Determination of bilirubin in plasma. Biochemistry. 1938; 297:281.
22. Vickers S, Duncan SA, Chen I-W, Rosegay A, Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab Dispos. 1990;18:138–145.
23. El-Serag HB, Everhart JE. Diabetes increases the risk of acute hepatic failure. Gastroenterology 2002;122:1822-8.
24. Liane P, Joel GR, Paula S, Gillian LB. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. *Canadian Medical Association Journal.* 2010;1-6. DOI:10.1503/cmaj.092144.
25. Veena N, Gincy TB, Prakash M, Chitralekha J, Soumya SR, Somayaji SN, Nelluri VM, Bairy KL. Hepatoprotective activity of aloe vera gel against paracetamol induced hepatotoxicity in albino rats. Asian J Phar Biol Res. 2011; 1(2): 94-98.
26. Omar MA, Amany AS, Nermeen MS. Effect of *Viscum album* on acute hepatic damage caused by carbon tetrachloride in rats Turk J Med Sci. 2010; 40 (3): 421-426.
27. Obata M. Mechanism of Anti-Inflammatory and Anti - Thermal Burn Action of Carboxypeptidase Aloe arborescence Miller, Netalensis Berger in Rats and Mice. Physiotherapy Research. 1993;7(Special Issues):530-533.
28. Shelton MS. *Aloe vera*, Its Chemical and Therapeutic Properties. International Journal of Dermatology. 1991;30:679-683.
29. Libor V. The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Frontiers in Pharmacology. 2012;3:1-7.
30. Breimer LH, Wannamethee G, Ebrahim S. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. *Clin Chem* 1995:41, 1504–1508.
31. Susie YY, John D, Shilpa R, Samuel H, Atul M, Aristidis V. Total serum bilirubin does not affect vascular reactivity in patients with diabetes. Vasc Med. 2009 May ; 14(2): 129–136. doi:10.1177/1358863X08098273.
32. Vítek L, Jirsa M, Brodanová M, Kalab M, Marecek Z, Danzig V, Novotný L, Kotal P. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis. 2002;160:449–456.
33. Djousse L, Levy D, Cupples LA, Evans JC, D'Agostino RB, Ellison RC. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. Am J Cardiol 2001;87:1196–1200.

34. Erdogan D, Gullu H, Yildirim E, Tok D, Kirbas I, Ciftci O, Baycan ST, Muderrisoglu H. Low serum bilirubin levels are independently and inversely related to impaired flow-mediated vasodilation and increased carotid intimamedia thickness in both men and women. Atherosclerosis 2006;184:431–437.
35. Yusuf L, Oladunmoye MK, Ogundare AO, Akinyosoye FA, Daudu OAY, Hassan GA. Antimicrobial and antioxidant properties of mistletoe (viscum album) growing on cola (cola nitida) tree in Akure North, Nigeria. Journal of Microbiology Research and Reviews. 2013;1(3):35-41.
36. Ademiluyi AO, Oboh G. Antioxidant properties of methanolic extracts of mistletoes (Viscum album) from cocoa and cashew trees in Nigeria. Afri J. Biotech. 2008;7(17):3138–3142.
37. Rajasekaran S, Sivagnanam K, Subramanian S. Antioxidant effect of Aloe vera gel extract in Streptozotocin-induced diabetes in rats. Pharmacol Rep. 2005;57(1):90-6.
38. Sergio M, Settimio S, Anita S, Ida P, Adelaide A, Saverio D. Protective Effect of *Aloe vera* (*Aloe barbadensis* Miller) on Erythrocytes Anion Transporter and Oxidative Change *Food and Nutrition Sciences*, 2012, 3, 1697-1702