

OCIMUM GRATISSIMUM ALLEVIATES DERANGEMENTS IN SERUM AND BILIARY BILIRUBIN, CHOLESTEROL AND ELECTROLYTES IN STREPTOZOTOCIN- INDUCED DIABETIC RATS

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39 **ABSTRACT**

40

41 The effects of oral administration of aqueous leaf extract of *Ocimum gratissimum* (OG) on
 42 biliary and serum bilirubin, cholesterol and electrolytes in streptozotocin (STZ)-induced diabetic
 43 Albino Wistar rats was studied. Eighteen rats were randomly assigned into 3 groups of 6 rats
 44 each for control, diabetic (DM) and diabetic-treated (DMT) groups. Diabetes mellitus was
 45 induced in the test groups (DM and DMT) by a single dose of STZ (65 mg/kg, i.p.). The
 46 phytoconstituents and median lethal dose of the plant extract was determined before
 47 administration. The extract was administered per oral to the DMT group at a dose of 1500 mg/kg
 48 body weight daily for 28 days. All the groups were fed normal rat chow and allowed water ad
 49 libitum. Biliary secretion was collected and assayed, biliary bilirubin was measured by
 50 colorimetric method, Sodium and potassium was determined using a flame photometer and
 51 Chloride was determined by end point calorimetric titration.

52 The result showed that serum cholesterol was significantly ($p=.001$) higher in the DM group
 53 compared to the control while the DMT group was significantly ($p=.001$) lower than the DM
 54 group. Serum conjugated and unconjugated bilirubin were significantly ($p=.05$, $p=0.01$) higher in
 55 the DM group compared to the control and DMT groups, with the DMT group significantly
 56 ($p=.01$) lower than the DM group. The serum electrolytes showed no significant change in K^+ .
 57 However, Cl^- and HCO_3^- were significantly ($p=.001$) reduced in the test group compared to the
 58 control. Na^+ was significantly raised in the DMT group compared to the control. We therefore
 59 conclude that OG was found to obviate the deranged biliary and serum bilirubin, cholesterol and
 60 electrolytes induced by diabetes.

61 **Key words:** *Ocimum gratissimum*, Diabetes mellitus, biliary secretion, serum electrolytes,
 62 cholesterol, bilirubin

63

64 **INTRODUCTION**

65 Airways of multisystem complications are known to be associated with DM; including
 66 alterations in the function of the hepatobiliary and renal systems. The **commonest** disease
 67 associated with DM in the biliary system, **is gallstones formation.** The pathogenesis of
 68 cholesterol gallstone formation is complex and many factors are involved, such as changes in
 69 bile composition (cholesterol, bile acids, phospholipids), changes in gall bladder motility and the
 70 presence of nucleation promotion factors. **Diabetic** have abnormal serum lipid profiles and
 71 increased biliary cholesterol secretion, resulting in increased cholesterol saturation of bile (1, 2).
 72 Elevated plasma insulin has been associated with an increased prevalence of gallstone disease (2)
 73 and may account for the strong association between, T2DM and gall stones.

74 Elevated levels of glucose, free fatty acids, hyperinsulinaemia and insulin resistance are
 75 the pathological basis for the development of diabetes complications. Glucotoxicity and
 76 lipotoxicity has been referred to as the diabetogenic effects of increasing blood levels of glucose
 77 and free fatty or increased cellular fat content respectively (3). Lipotoxicity is a common feature
 78 in diabetes mellitus and it has a strong association with obesity. It is also believed that excess
 79 fuel in form of fat may be responsible for rising blood glucose concentration (3). Several
 80 explanations for lipotoxicity have emerged. An explanation for lipotoxicity involves the Randle
 81 (glucose – fatty acid) cycle (4). According to this hypothesis, fatty acid glucose oxidation can be
 82 thought of as competitive in the sense that excess fat metabolism impairs the oxidation of
 83 glucose. The formation of acetyl coenzyme A (CoA), a product of both glucose and fat oxidation
 84 mediates this process of glucose impairment. Acetyl CoA inhibits glucose use by allosteric
 85 inhibition of pyruvate dehydrogenase, the enzyme complex used for pyruvate conversion to
 86 acetyl CoA, the last step in delivery of glycolytic fuel to mitochondria (3, 4).

87 Other explanations for lipotoxicity involve fatty acid inhibition of cellular glucose entry
 88 through the inhibition of one or more steps in the insulin-signalling cascade. Free fatty acids may
 89 impair GLUT4 gene expression in muscle and fat (5). In addition, free fatty acids decrease
 90 glucose conversion into glycogen for storage, while also causing increase hepatic glucose output
 91 from gluconeogenesis (5, 6). There seems to be a kind of competition between glucose and fat in
 92 that not only does fat oxidation impair glucose uptake and metabolism, but glucose itself can
 93 inhibit fat utilization (3).

Alterations in the function of the liver, biliary system and exocrine pancreas are common in DM. However, in contrast to other systemic disorders with GI involvement; symptoms originating from these organs are less prominent, apart from biliary colic due to gallstones.

Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction (7). Electrolyte imbalance in diabetes is primarily a result of elevated blood glucose. With hyperglycemia, the body tries to rid itself of the excess blood glucose by increasing urinary output. Increased urination produces water and electrolyte loss, which then upsets the body's balance of electrolytes. The balance is especially disturbed between sodium and potassium (8). Electrolyte imbalance resulting from kidney failure, dehydration, fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (7).

Ocimum gratissimum is one of the species from the genus *Ocimum*. It is commonly called African basil or shrubby basil (9). OG is a genus of about thirty five species of aromatic annual and perennial herbs and shrubs in the family Lamiaceae. It is widely distributed in tropical and warm temperate regions. It has a strong taste with characteristic flavour. It is used commonly by the various tribes of Nigeria for nutritional and medicinal purposes. It is called “Nton” in Ibibio/Efik, “Efinrin” in Yoruba, “Daidoya” in Hausa and “Nchonwu” in Igbo.

Phytochemical analysis revealed important constituents as tannins, alkaloids, saponins, flavonoids and phenolic compounds (10). The volatile aromatic oil from the leaves of this plant consists mainly of thymol (32-65 per cent) and eugenol: it also contains xanthenes, terpenes, and lactones (11). Also citral, ethyl cinnamate, geraniol and linalool have also been extracted from this oil (12).

The hypoglycemic properties of OG claimed by Nigerian traditional herbal medicine practitioners had been investigated and confirmed to be true (13, 14) but there is paucity of data on the effect of DM on biliary and serum bilirubin, cholesterol and electrolytes, these study therefore set out to determine if DM affect these parameters and if OG could ameliorate these effects (if any).

123 MATERIALS AND METHODS

124 Plant materials and Preparation of aqueous extract

125 The leaves of *Ocimum gratissimum* were obtained from the University of Calabar
 126 Botanical Garden and identified by the Chief Herbarium Officer of Botany Department of
 127 University of Calabar. The fresh leaves were rinsed with water to remove sand and debris and
 128 then allowed to drip off water. The leaves were then dried under shade for two days and then
 129 transferred into Astell Hearson Oven and dried at a temperature range of 40 – 45°C.

130 The dried leaves were then ground in an electric blender into fine powder to give a gram weight
 131 of 527grams. This 527g weight was soaked in 2.65 liters of water (distilled water) and allowed
 132 over night for about 15 hours and stirred at interval. The mixture was filtered using a satin mesh
 133 material and the final filtrate was gotten by using Whatman's filter paper size 1. The final
 134 filtrate was dried in the Astell Hearson Oven at 45°C to obtain a brown gummy paste. A mettler
 135 P163 electronic weighing balance was used to weigh the gummy paste before stock solution was
 136 prepared. The stock solution of the extract was prepared by dissolving 15gm of extract in 10ml of
 137 water to give a concentration of 1500mg/ml. The stock solution was labeled appropriately and
 138 refrigerated at 4°C until required for use. The median lethal dose (LD₅₀) of the plant extract was
 139 determined by method of Lorke (1983) (15).

140 Determination of phytoconstituents

141 The phytoconstituents of the extracts was determined and were screened for the presence
 142 of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones and cardiac glycosides
 143 as described by Trease and Evans (1984) (16) and Sofowora (1984) (17).

144 Animals Preparation, experimental groupings and treatment

145 Eighteen rats were used for the study, the animals were divided into three groups and
 146 were assigned randomly into each group which was made up of Six (6) rats each and housed in
 147 cages assigned to them.

148 The first group was made up of the control animals which were fed with normal rat chow
 149 (feed). The second group contained streptozotocin induced diabetic rat which were left
 150 untreated. The third group of animals contained the test group which were streptozotocin
 151 induced diabetic rats treated with aqueous leaf extract of *Ocimum gratissimum*. The experimental

procedures involving the animals and their care were in line with the approved guidelines by the local research and ethical committee.

Induction of diabetes mellitus

Diabetes mellitus was induced by a single injection of 65mg/kg streptozotocin. The injection was given intraperitoneally. The state of diabetes was observed after 48 hours by the symptoms of *polyuria* and glucosuria and this state was confirmed using uristic test strip (Bayer Health Care LLC, US). Also, the blood glucose level was tested 1 week after induction using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics (GmbH, Germany) and ACCU-CHECK Advantage II test strips.

Extract administration and observation

One week after induction of diabetes, extract was administered per oral to the DMT group at a dose of 1500 mg/kg body weight daily for 28 days. Administration was facilitated by the use of a syringe and Orogastic tube.

Collection of biliary secretion

Biliary secretion was collected by the method of Vickers *et al*, (1990) (18). The rats were starved for 12 hours prior to the time of experiment. The animals were weighed and anaesthetized by intraperitoneal administration of sodium thiopentone (6mg/100g body weight), and were quickly pinned to a dissecting board for a tracheostomy performed to clear the airway for easy breathing. The stomach was opened along the linea alba to prevent bleeding. A laparotomy was performed and the liver lobes deflected anterolaterally to expose the common bile duct. The common bile duct was then Cannulated with a portex Cannula (0.5mm in diameter) after a small incision was made. The Cannula was held in place with a thread tied round the bile duct. The bile content was collected at 3 hours interval for each group studied.

Determination of biliary bilirubin

Biliary bilirubin was measured by colorimetric method as described by Jendrassik and Grof, (1938) (19) and later Sherlock (1951) (20).

Determination of biliary electrolytes: sodium and potassium, bicarbonate, chloride

Sodium and potassium in bile were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Plasma carbon dioxide (CO₂) was measured by the modified method of Forrester *et al* (1976) (21). Biliary Chloride was determined by end point calorimetric titration following the method of Kolthoft *et al* (1967) (22).

Extraction of cholesterol from bile secretion

Cholesterol was determined using the method of Allain *et al* (1974) (23).

Statistical analysis

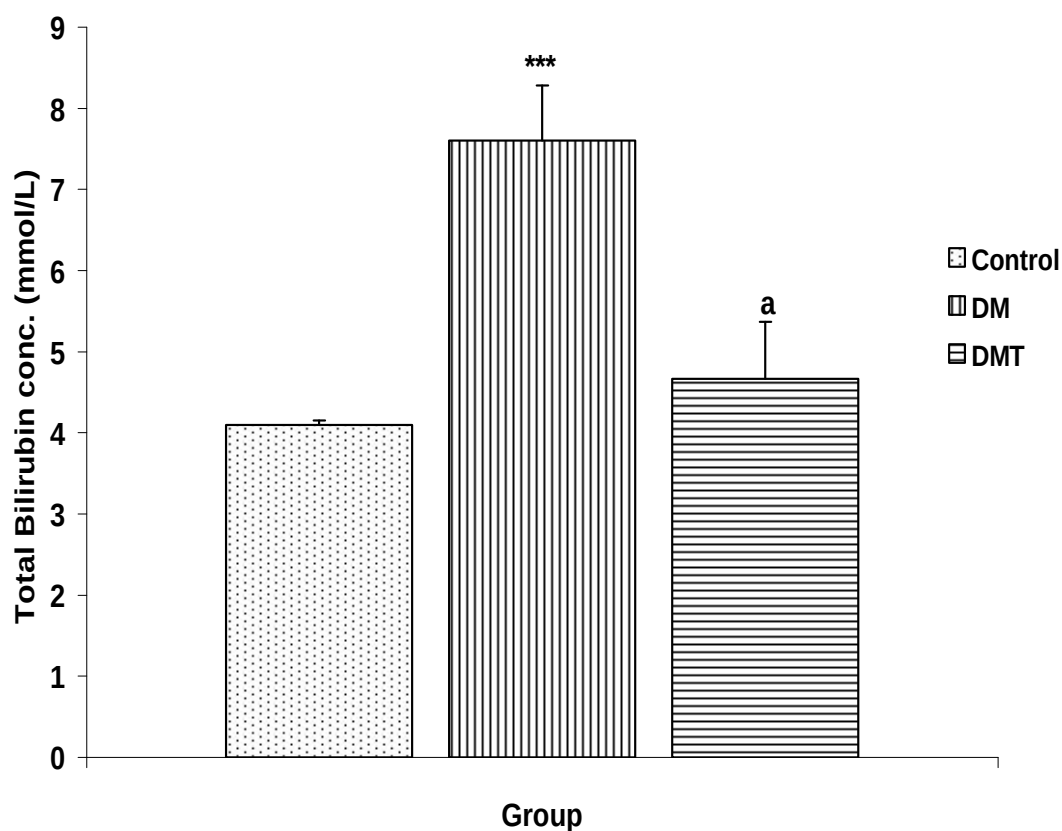
All results are presented as mean $\pm$ standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the least significant difference (LSD) procedure for significant F values, (P=.05) was considered significant. A Computer software SPSS and Excel Analyzer was used for the analysis.

3. RESULTS

3.1 Serum cholesterol, total bilirubin, conjugated bilirubin and unconjugated bilirubin in the different experimental groups

3.1.1 Serum cholesterol level in the control, DM and DMT groups of experimental rats

The mean cholesterol concentrations were: 0.87 ± 0.013 , 1.37 ± 0.091 and 0.9 ± 0.036 mmol/L respectively in the control, DM and DMT groups. They were all significantly different. The DM was significantly higher ($P=.001$) than the control, and the DM was significantly higher ($P=.001$) than the DMT, fig.1.



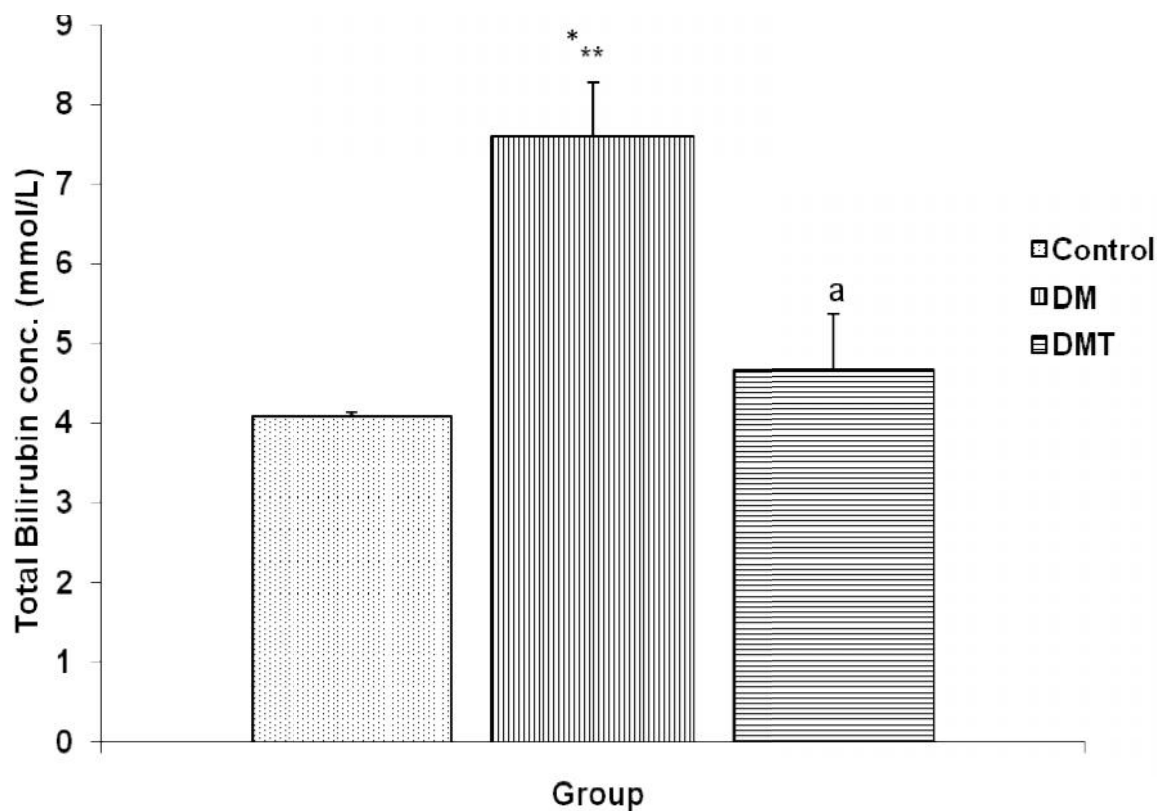
***P=.001 vs control, a = P=.05 vs DM.

Values are mean ± SEM, n = 6.

FIG. 1: Comparison of total serum cholesterol (TC) concentration in the different experimental groups.

3.1.2 Estimated total serum bilirubin in the various experimental groups of rats

The mean values for the total serum bilirubin concentration in the control, DM and DMT groups were: 4.1 ± 0.044, 7.6 ± 0.948 and 4.7 ± 0.678 respectively. The DM was significantly higher (P=.001) than the control; while the DMT was significantly lower (P < 0.05) than the DM, fig. 2.



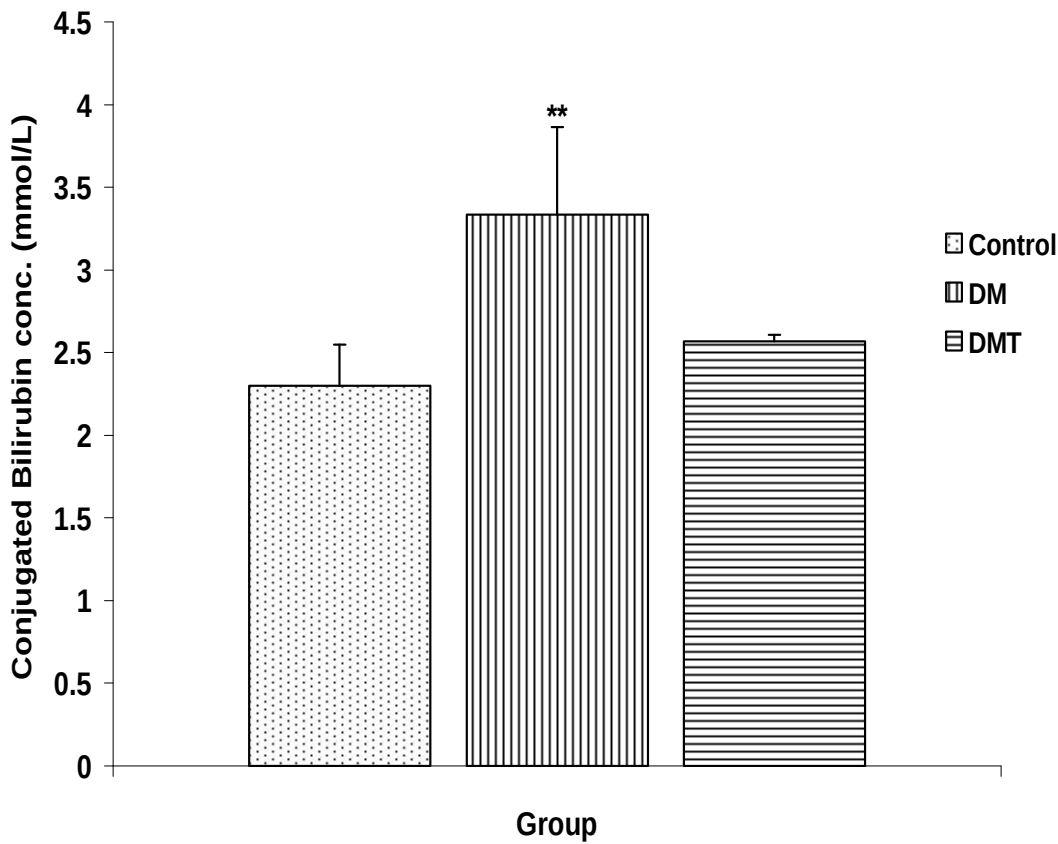
***P=.001 vs control; a = P=.05 vs DM.

Values are mean ± SEM, n = 6.

FIG. 2: Comparison of total serum bilirubin concentration in the different experimental groups.

3.1.3 Estimated serum conjugated bilirubin levels in the control, DM and DMT experimental groups of rats

The mean values of serum conjugated bilirubin were: 2.3 ± 0.44 , 3.3 ± 0.25 and 2.6 ± 0.53 mmol/L for control, DM and DMT respectively. The DM group was significantly higher (P=.01) than the control, fig.3



**P=.01 vs control.

Values are mean ± SEM, n = 6.

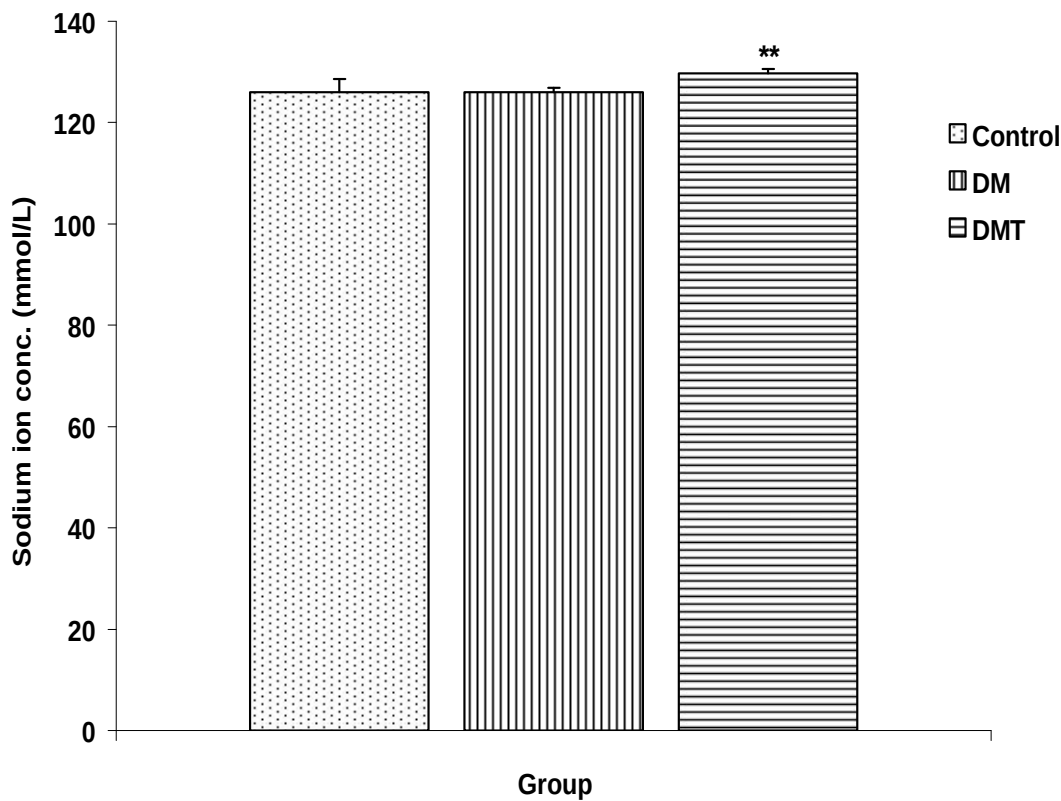
FIG. 3: Comparison of serum conjugated bilirubin concentration in the different experimental groups.

3.2 Serum electrolytes in the various experimental groups of rats

The electrolytes investigated include: Na⁺, K⁺, Cl⁻, and HCO₃⁻.

3.2.1 Serum sodium ion concentration in the control, DM and DMT experimental groups of rats

The mean Na⁺ concentrations were 126 ± 0.894, 126 ± 2.632, and 129 ± 0.918mmol/L in the control, DM and DMT groups respectively. The DMT group was significantly (P=.01) higher than the control group. (Fig. 4)

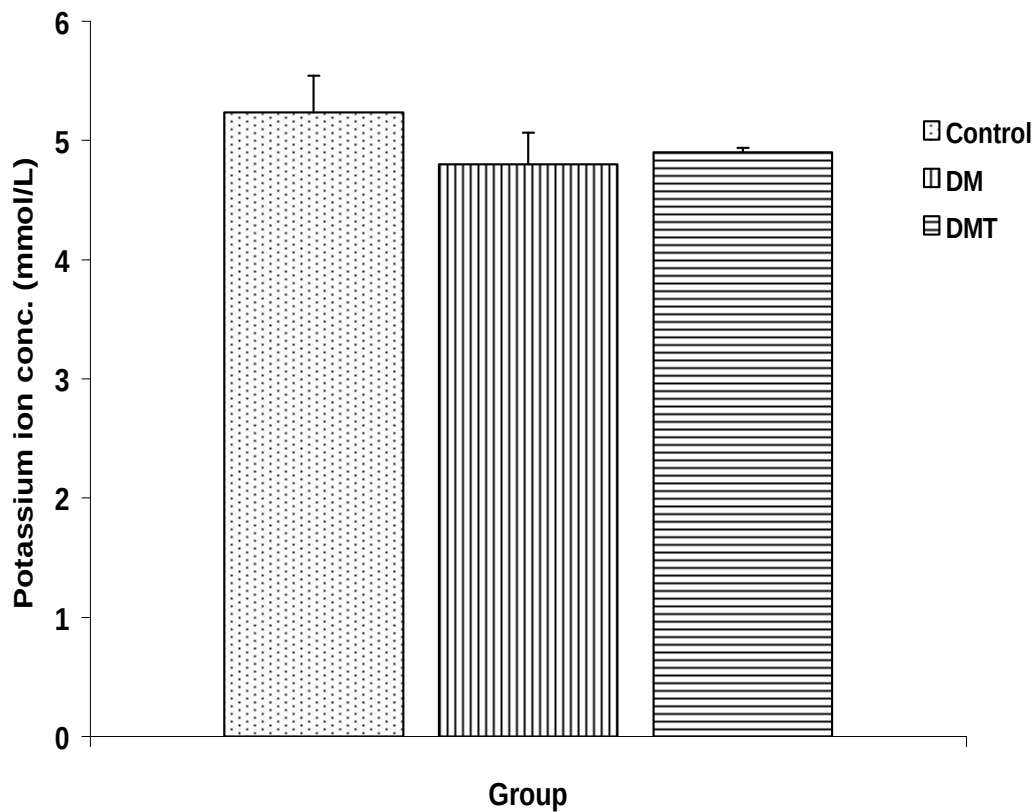


**P=.01 vs control. Values are mean ± SEM, n = 6.

Fig. 4: Comparison of serum sodium ion concentration in the different experimental groups.

3.2.2 Serum potassium ion concentration in the control, DM and DMT experimental groups of rats

The mean K^{+} concentration in the control, DM and DMT groups were: 4.9 ± 0.44 , 5.2 ± 0.31 , and 4.8 ± 0.26 mmol/L respectively. There were no significant differences between the groups, fig. 5

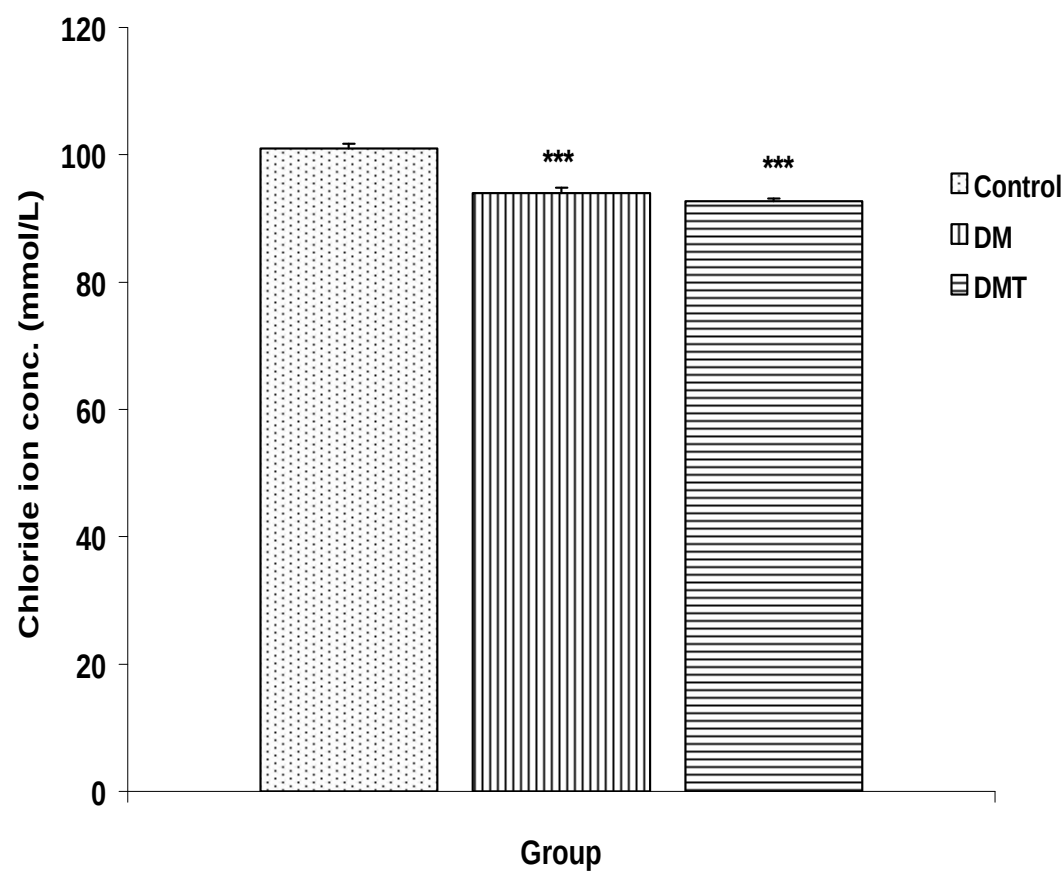


Values are mean $\pm$ SEM, n = 6.

Fig. 5: Comparison of serum potassium ion concentration in the different experimental groups.

3.2.3 Serum chloride ion concentration in the control, DM and DMT experimental groups of rats

The mean Cl^- concentrations in these groups were: 101 ± 0.45 , 94 ± 0.73 and $92 \pm 0.84\text{mmol/L}$ respectively. The test groups (DM and DMT) concentrations were significantly ($P=.001$) lower than the control group, fig.6

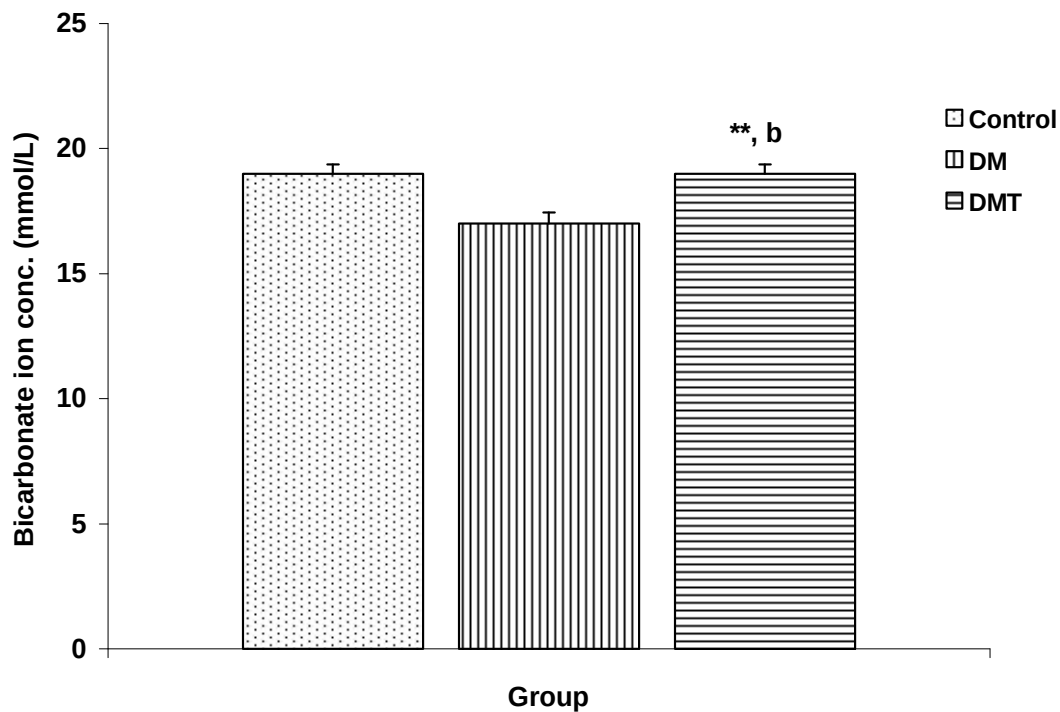


*** $P=.001$ vs control. Values are mean $\pm$ SEM, $n = 6$.

Fig. 6: Comparison of serum chloride ion concentration in the different experimental groups.

3.2.4 Serum bicarbonate ion concentration in the various experimental groups of rats

The mean HCO_3^- concentration in the control, DM and DMT groups were: 19 ± 0.45 , 17 ± 0.37 , and 19 ± 0.37 respectively. There were significant differences between the groups. The DMT was significantly ($P=.01$) lower than the control, while the DMT was significantly higher ($P=.01$) than the DM group, fig.7



**P=.01 vs control; b = P=.01 vs DM

Values are mean $\pm$ SEM, n = 6.

Fig 7: Comparison serum of bicarbonate ion concentration in the different experimental groups.

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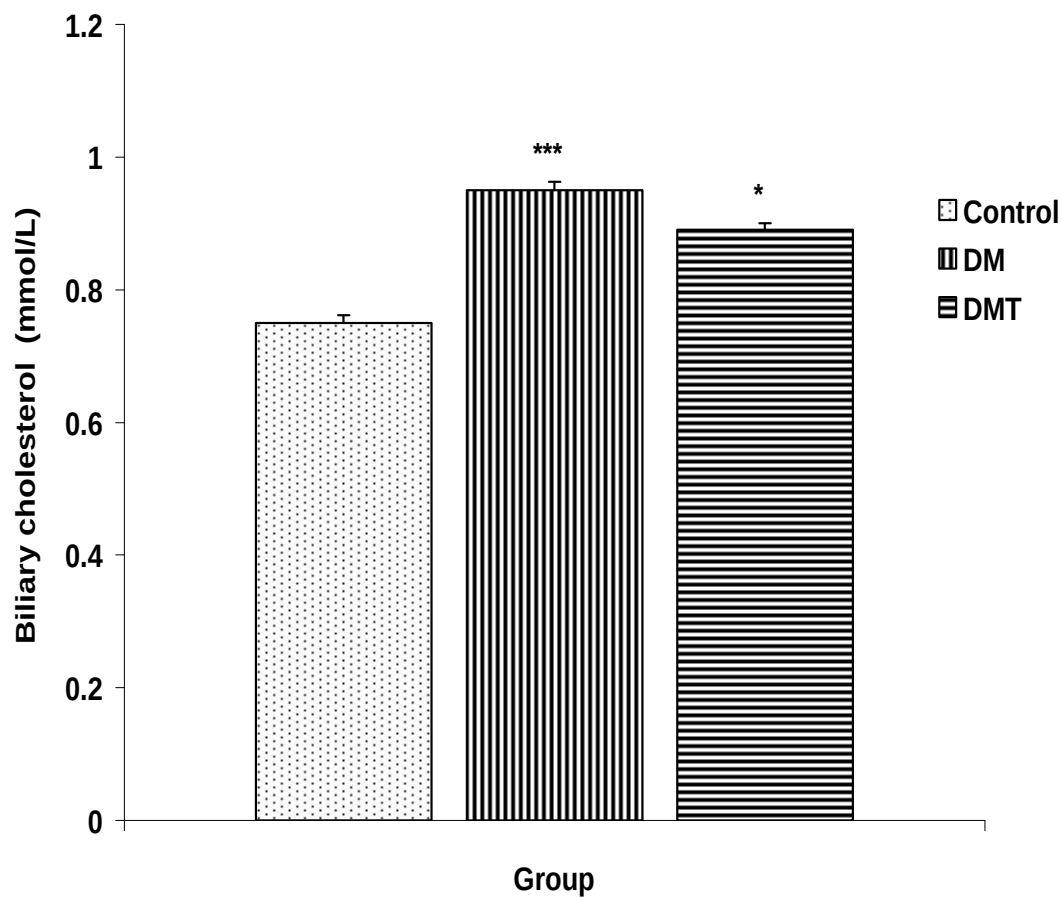
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323 **3.3 Biliary cholesterol, total bilirubin, conjugated bilirubin and unconjugated bilirubin**
 324 **in the different experimental groups of rats**

325 **3.3.1 Biliary cholesterol concentration in the different experimental groups of rats**

326 The mean biliary cholesterol concentration in the control, diabetic control and diabetic
 327 treated groups were 0.63 ± 0.01 , 0.95 ± 0.10 and 0.89 ± 0.01 mmol/L respectively. The
 328 cholesterol concentration in the diabetic control was significantly higher ($P < 0.05$) when
 329 compared with the control. However, the cholesterol concentration in the diabetic-treated was
 330 significantly higher ($P=.001$) than in the control, but was also not significantly higher than that in
 331 the diabetic control group (fig. 8).



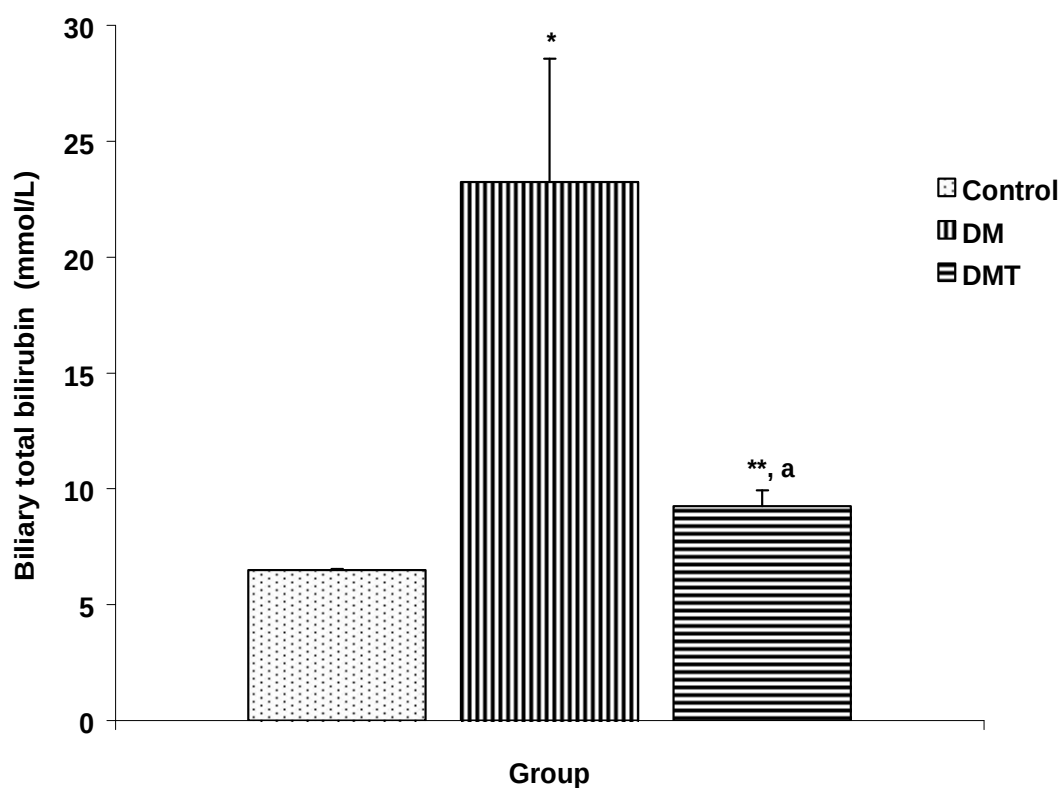
*P=.05, ***p=.001 vs control

Values are mean ± SEM, n = 6.

Fig 8: Comparison of biliary cholesterol concentration in the different experimental groups.

3.3.2 Biliary total bilirubin concentration in the different experimental groups of rats

The mean total biliary bilirubin concentration in the diabetic control (23.23 ± 5.11 mmol/L) was significantly higher ($P < 0.05$) when compared with the control group (6.30 ± 0.57 mmol/L). However, the mean total bilirubin concentration in the diabetic-treated (9.25 ± 0.18 mmol/L) was significantly higher ($P=.001$) than that in the control group, but was also significantly lower ($P < 0.05$) when compared with the diabetic control group (fig. 9)



*P=.05, **P=.001 vs control; a = P=.05 vs DM.

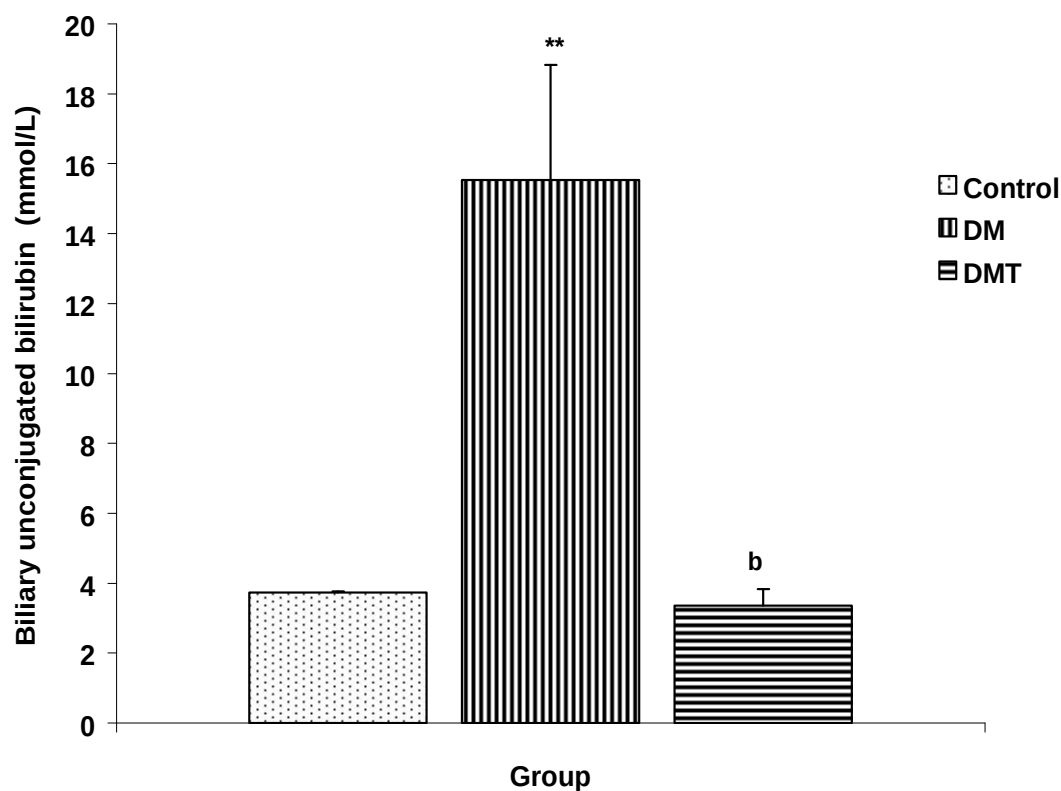
Values are mean ± SEM, n = 6.

Fig 9: comparison of biliary total bilirubin concentration in the different experimental groups

3.3.3 Biliary conjugated bilirubin concentration in the different experimental groups of rats

The mean biliary conjugated bilirubin concentration in the control, diabetic control and diabetic treated were 3.73 ± 0.44 , 15.53 ± 3.08 and 3.33 ± 0.45 mmol/L respectively. The result showed that the conjugated bilirubin concentration in the diabetic control group was significantly higher (P=.001) when compared with the control group. However, the conjugated bilirubin

concentration in the diabetic treated group was significantly lower ($P=.001$) than that in the diabetic control group (fig. 10).



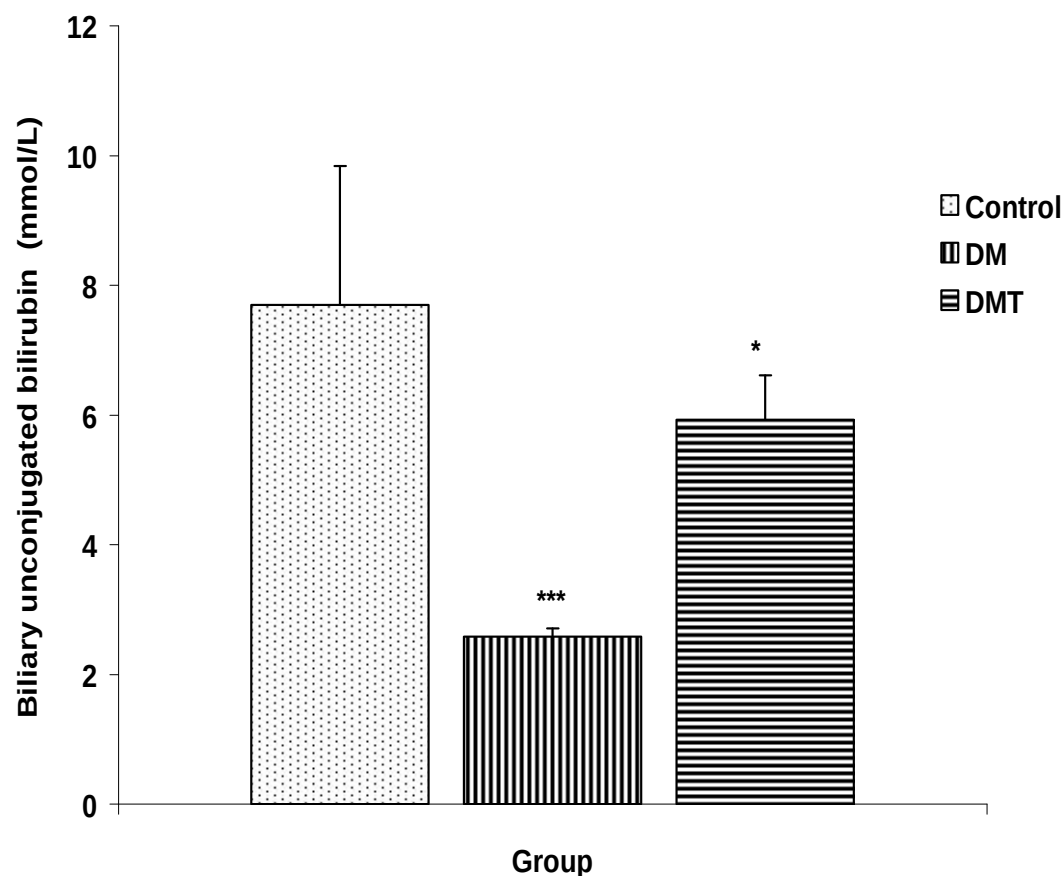
$**P=.001$ vs control; $b = P=.01$ vs DM.
 Values are mean $\pm$ SEM, $n = 6$.

Fig 10: Comparison of biliary conjugated bilirubin concentration in the different experimental groups.

3.3.4 Biliary unconjugated bilirubin concentration in the different experimental groups of rats

The mean biliary unconjugated bilirubin concentration in the diabetic control group was 7.70 ± 2.10 mmol/L. This was significantly higher ($P=.05$) than that in the control group (2.58 ± 0.13 mmol/L). The mean unconjugated bilirubin concentration in the diabetic treated group (5.93

378 $\pm 0.50\text{mmol/L}$) was significantly higher ($P=.001$) than that in the control group. There was no
379 significant difference between the diabetic control and the diabetic treated groups (fig. 11).



380
381 * $P=.05$, *** $P=.001$ vs control
382 Values are mean $\pm$ SEM, $n = 6$.
383 **Fig 11: Comparison of biliary unconjugated bilirubin concentration in the different**
384 **experimental groups.**

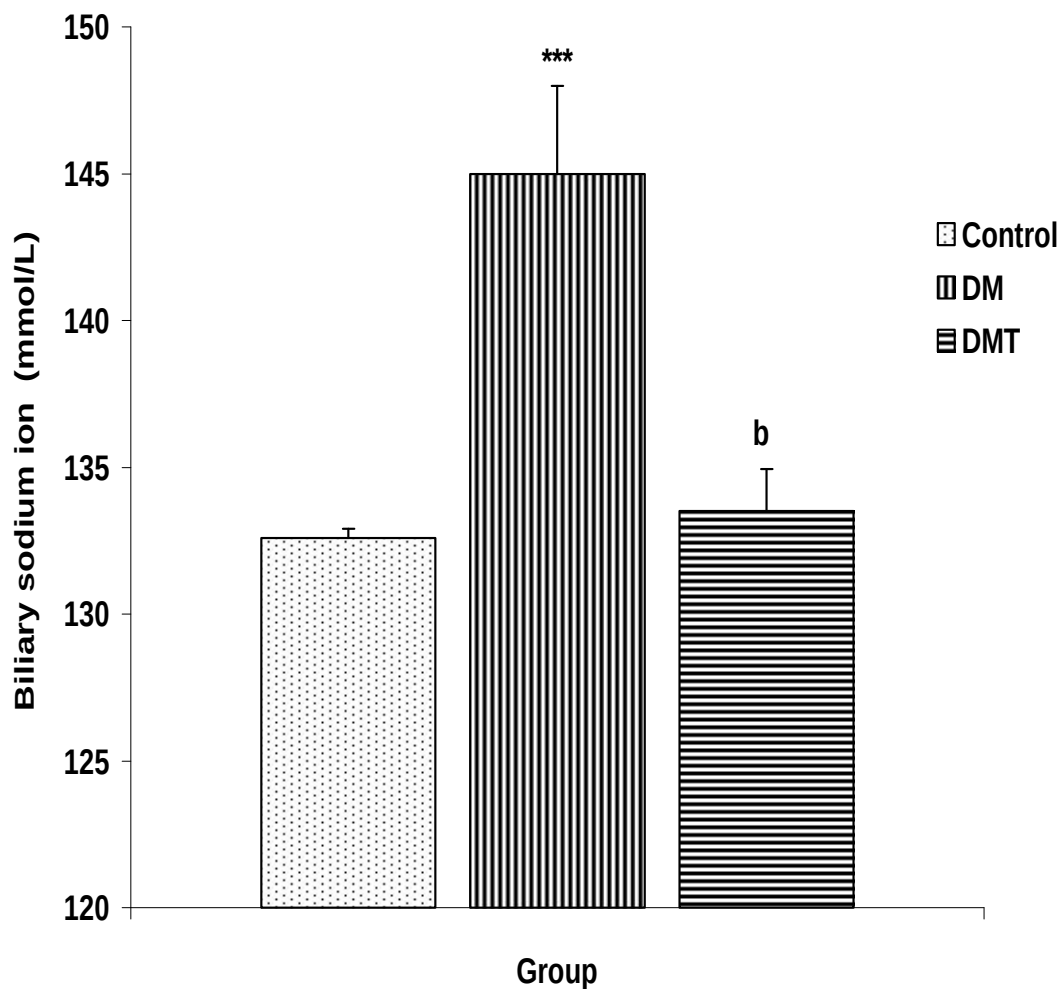
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389 **3.4 Biliary electrolytes in the various experimental groups of rats**

390 **3.4.1 Biliary sodium ion (Na^+) concentration in the different experimental groups of rats**

391 The mean Sodium ion (Na^+) Concentration in the control, Diabetic control and Diabetic
392 treated groups were 132.5 ± 0.29 , 148.0 ± 2.58 and $133.5 \pm 1.44\text{mmol/L}$ respectively. The result
393 shows that Na^+ concentration in the Diabetic control group was significantly higher ($P=.001$)

394 than in the Control group. The Diabetic treated group, showed a significant decrease ($P=.01$) in
395 Na^+ concentration when compared with the Diabetic Control Group (fig. 12).

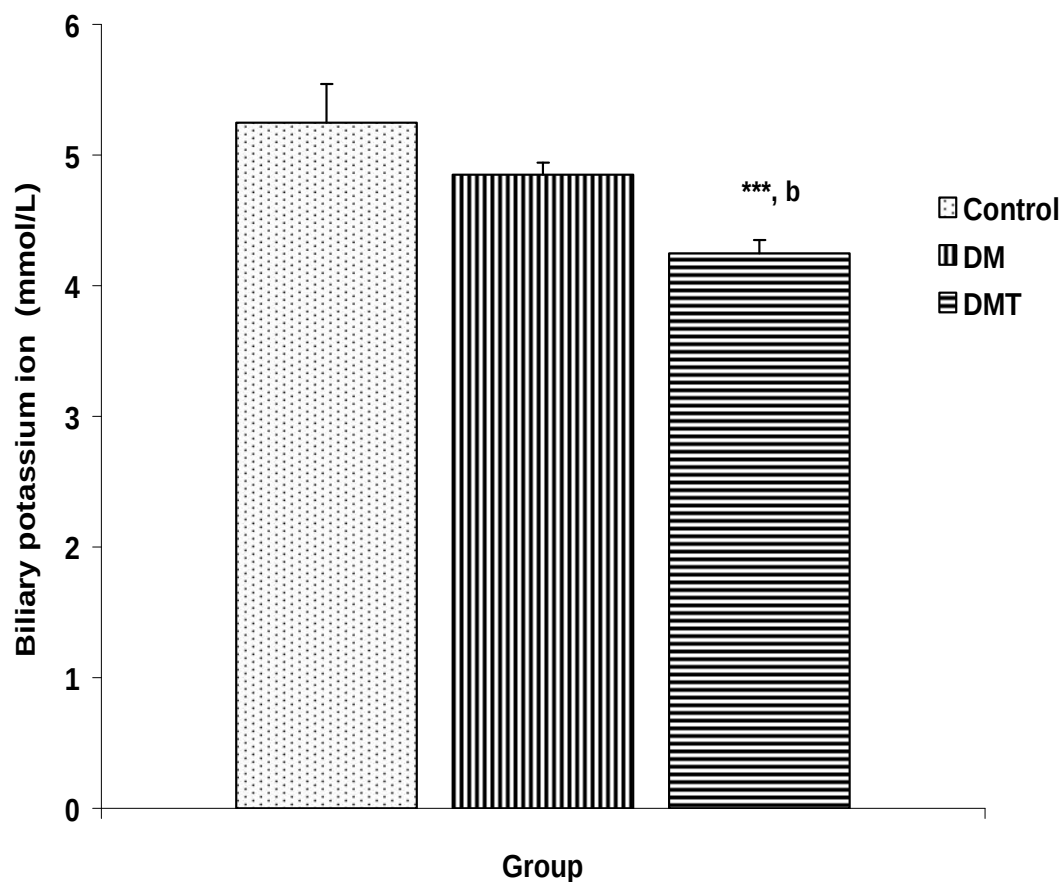


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398 *** $P=.001$ vs control; b = $P=.01$ vs DM.
399 Values are mean $\pm$ SEM, n = 6.

400 **FIG. 12: Comparison of biliary sodium ion levels in the different experimental groups.**

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404 **3.4.2 Biliary potassium ion (K^+) concentration in the different experimental groups of**
405 **rats**

406 Bile potassium concentration in diabetic control group ($5.25 \pm 0.29\text{mmol/L}$) was not
407 significantly different when compared to the control group ($4.85 \pm 0.09\text{mmol/L}$). However, the
408 K^+ concentration in the diabetic treated group ($4.05 \pm 0.10\text{mmol/L}$) was significantly lower than
409 in the control ($P=.001$) and diabetic control ($P=.001$) respectively (fig. 13).



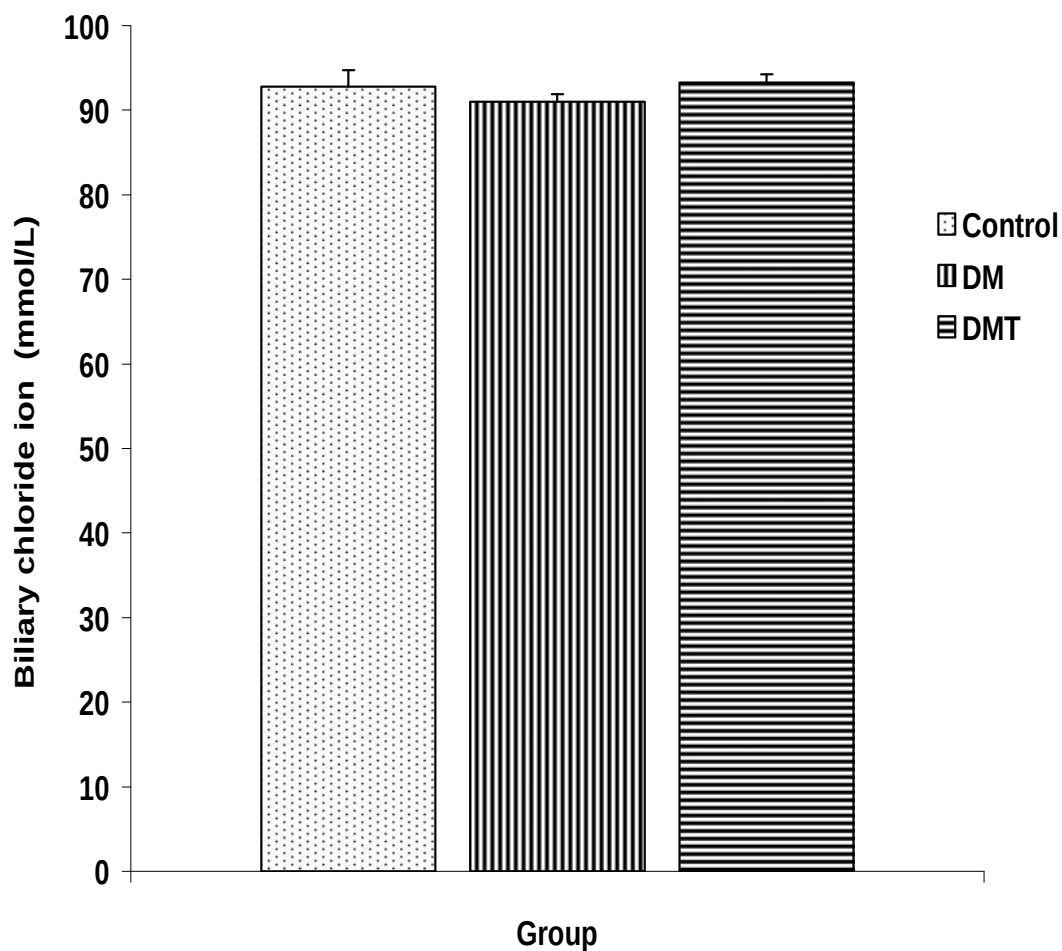
***P=.001 vs control; b = P=.01 vs DM.

Values are mean $\pm$ SEM, n = 6.

FIG. 13: Comparison of biliary potassium ion levels in the different experimental groups.

3.4.3 Biliary chloride ion (Cl⁻) concentration in the different experimental groups of rats

The mean bile chloride ion concentration in the control, diabetic control and diabetic treated were 92.75 ± 0.48 , 91.00 ± 1.29 and 93.25 ± 0.48 mmol/L respectively. There was no significant difference amongst the groups (fig. 14).



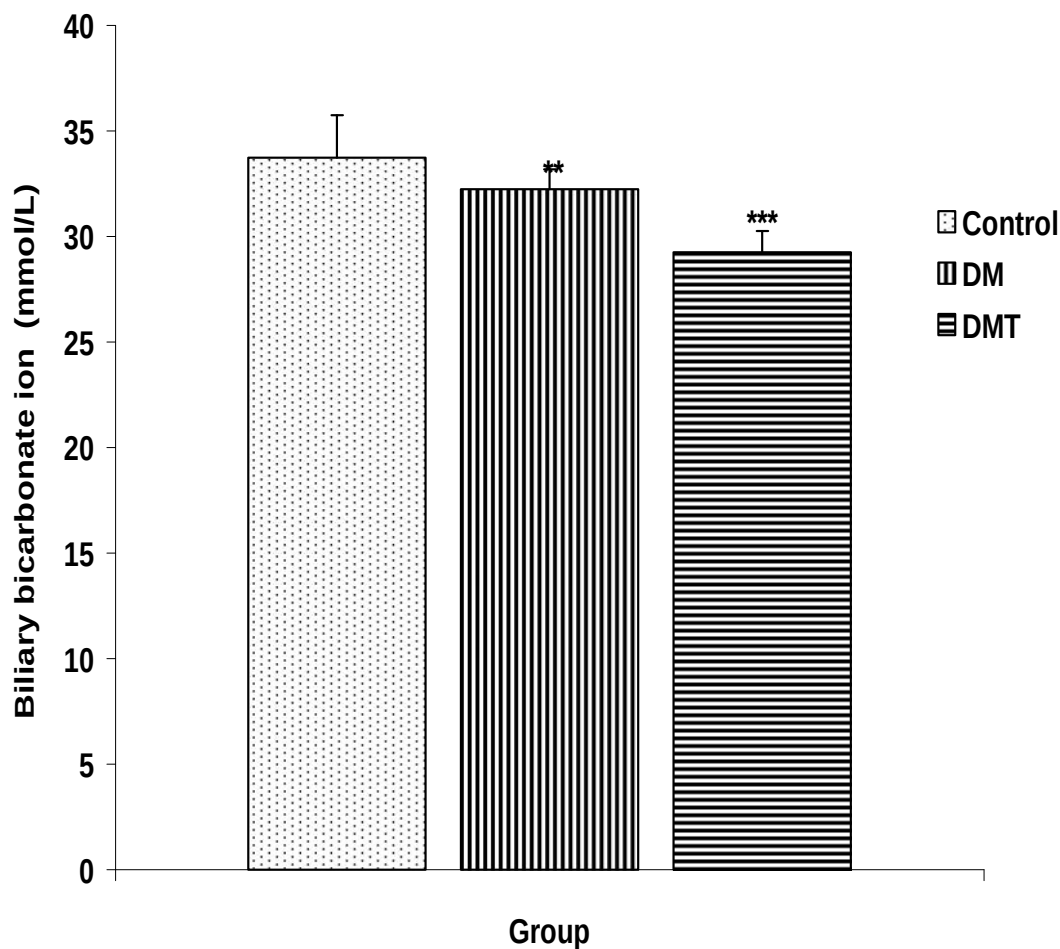
Values are mean $\pm$ SEM, n = 6.

FIG. 14: Comparison of biliary chloride ion levels in the different experimental groups.

3.4.4 Biliary bicarbonate (HCO_3^-) ion concentration in the different groups of rats

The mean bile bicarbonate ion (HCO_3^-) concentration in the diabetic control group ($33.75 \pm 1.03\text{mmol/L}$) and diabetic-treated group ($32.25 \pm 0.48\text{mmol/L}$) were significantly higher ($P=.001$) when compared with that in the control group ($29.25 \pm 0.48\text{mmol/L}$). There was no

significant difference in HCO_3^- concentration in diabetic-treated group when compared with the diabetic control group (fig. 15).



*** P=.001, **P=.01 vs control.

Values are mean $\pm$ SEM, n = 6.

FIG. 15: Comparison of biliary bicarbonate ion levels in the different experimental groups.

4. DISCUSSION

Serum and biliary bilirubin, cholesterol and electrolytes

Total conjugated and unconjugated bilirubin in the serum and bile were raised in the DM and DMT test groups compared to the control group. There was a significant reduction of this level in the DMT group when compared to the DM group. This connotes that OG reduces bilirubin levels raised in diabetics. The pattern of increment in the various forms of bilirubin estimated in this study were similar in both serum and bile; pointing to the possibility of extrahepatic involvement in the pathologic process involved in the rise. Hyperbilirubinaemia without any abnormality of other liver function tests may result from increased bilirubin production, as in haemolysis or ineffective erythropoiesis, or from inability to transport bilirubin across the liver as in Gilbert's syndrome (24).

Irrespective of the pathologic site resulting in the bilirubin levels increase in DM, this research has demonstrated the fact that OG tends to reverse the abnormality as seen in the significant reduction of the bilirubin levels in the DMT group. This is also an indicator that OG may possess some hepato-protective properties.

Serum and biliary cholesterol levels showed a related increase in the DM and DMT groups, when compared with the control group. The cholesterol estimate in both serum and bile were lower in the DMT group compared to the DM group. It is reported that diabetics have abnormal serum lipid profiles and increased biliary cholesterol secretion, resulting in increased cholesterol saturation of bile (1, 2). It therefore appears from the result of this study that OG treatment improves the abnormal serum lipid profiles, thus leading to reduction in both serum and biliary cholesterol levels. It is possible that this may be related to the enhanced pancreatic exocrine activity of OG which increased pancreatic amylase and lipase secretion.

Serum electrolytes levels were predominantly decreased except for sodium (Na^+) in the test groups compared to the control. While all the levels of bile electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) estimated in this study were generally increased in the test groups when compared to the control group.

The kidney plays a crucial role in electrolyte regulation. Between the test groups, serum electrolytes level were higher (near normal) in the DMT group than DM group except chloride ion. Also bile electrolytes level was lower in the DMT group than DM group except potassium ion. There were no significant changes in the bile chloride ion levels for control and test groups. Shahid and Mahaboo, (2008) (25) had reported the progressive trends in electrolyte

488 abnormalities in DM leading to end stage renal disease along with the abnormality of their chief
489 transport mechanism.

490 There is increasing evidence that reactive oxygen species (ROS) play a major role in the
491 development of diabetic complications. Ha *et al*, (2008) (26) had reported that oxidative stress is
492 increased in DM, and the over production of ROS in DM is a direct consequence of
493 hyperglycemia. He also noted that various types of vascular cells including renal cells are able to
494 produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron
495 gradient play roles in hyperglycemia-induced ROS generation (26). In addition to their ability to
496 directly inflict macromolecular damage, ROS can function as signaling molecules to mediate
497 hyperglycemia-induced activation of signal transduction of profibrotic genes in the kidney
498 leading to renal disease (26). The resulting renal disease may affect electrolyte handling!
499 Conventional and catalytic antioxidants have been shown to prevent or delay the onset of
500 diabetic nephropathy, by opposing the activity of ROS (26). The fact that the serum electrolyte
501 levels in the DMT group were closer to the control suggests that OG may possess antioxidant
502 agent capable of abolishing or delaying the activity of ROS. Thus, ameliorating the diabetic
503 nephropathy and enhances renal functions including electrolyte regulation.

504 Insulin deficiency causes drift of K^+ ion out of cells leading to hyperkalemia (27). The
505 significant increase in K^+ concentration in bile in the DMT group of experimental rats compared
506 to the control and DM group supports the hypothesis that the hypoglycemic effect of OG may
507 not be related to increase insulin secretion from the pancreatic islets. The overall rise in bile
508 electrolyte concentration compared to serum electrolytes further buttresses the cholagogue action
509 of OG. It is known that glycosuria (a cardinal feature of DM) causes dehydration via glucose
510 osmotic diuresis. This dehydration is accompanied with severe loss of electrolyte including
511 Na^+ , K^+ , Ca^{2+} , Cl^- and PO_4^- (28, 29). This study also collaborate these previous reports, with a
512 markedly reduced serum electrolytes levels in the DM and DMT groups.

513

514

515 **5. CONCLUSION** 

516 Derangement in biliary and serum bilirubin, cholesterol and electrolytes were observed in STZ-
517 induced diabetic rats. Treatment with OG was found to obviate this abnormality.

518 **COMPETING INTERESTS**

519 Authors have declared that no competing interests exist.

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521 **REFERENCES**

- 522 1. De Santis, A., Attili, A.F. & Corradini, S.G. (1997). Gallstones and diabetes mellitus: a
523 case control study in a free living population sample. *Hepatology* 25: 787-790.
- 524 2. Laakso, M., Suhonen, M., Julkunen, R. & Pyorola, K. (1990). Plasma insulin, serum
525 lipids and lipoproteins in gallstone disease in non-insulin dependent diabetic subjects: a
526 case control study. *Gut* 31: 344-347.
- 527 3. Sivitz, W. I. (2001). Lipotoxicity and glucotoxicity in type 2 diabetes: effect on
528 development and progression. *Postgraduate Medicine* 109: 55 – 64.
- 529 4. Randle, P.J., Priestman, D. A. & Mistry, S.C. (1994). Glucose-fatty acid interactions and
530 the regulation of glucose disposal. *Journal of Cellular Biochemistry* 55 (Suppl): 1 – 11.
- 531 5. Boden, G. (1997). Role of fatty acids in the pathogenesis of insulin resistance and
532 NIDDM. *Diabetes* 46: 3 – 10.
- 533 6. Kelley, D.E. & Mandario, L.J. (2000). Diabetes, oxidative stress, and platelet activation.
534 *Circulation* 99: 189-191.
- 535 7. Rao G. M. (1992). Serum electrolytes and osmolality in diabetes mellitus. *Indian J Med*
536 *Sci*; 46 (10):301-303.
- 537 8. Lisa Michael, 2011. Available at: [http://www.livestrong.com/article/353569-electrolyte-](http://www.livestrong.com/article/353569-electrolyte-imbalance-in-diabetes)
538 [imbalance-in-diabetes](http://www.livestrong.com/article/353569-electrolyte-imbalance-in-diabetes).
- 539 9. Iwu, M.M. (1993). *Handbook of African Medicnal Plants*. CRC press, New York. Pp
540 214-215.
- 541 10. Afolabi, C., Akinmoladun, E. O., Ibukun, I., Emmanuel, A., Obuotor, E. M. & Farombi,
542 E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves
543 of *Ocimum gratissimum*. *Scientific Research & Essay* 2 (5):163-166.
- 544 11. Ezekwesili, C.N., Obiora, K.A., & Ugwu, O.P. (2004). Evaluation of Anti-Diarrhoeal
545 property of Crude Aqueous Extract of *Ocimum gratissimum* L.(Labiatae) in Rats.
546 *Biokemistri Nigerian Society for Experiment Biology* 16(2): 122-131.
- 547 12. Sulistiarini, D.L., Oyen, P.O. & Ngugen, X.D. (1999). *Ocimum gratissimum* L. In: *Plant*
548 *Resource of South-East Asia*. No.19: Essential oil Plants. Prosea Foundation. Bogor
549 Indonesia. 140-142.

550

13. Okon U. A; Owo D.U; Udokang N.E; Udobang J.A.; Ekpenyong C.E. (2012). Oral Administration of Aqueous Leaf Extract of *Ocimum Gratissimum* Ameliorates Polyphagia, Polydipsia and Weight Loss in Streptozotocin-Induced Diabetic Rats. *American Journal of Medicine and Medical Sciences*; 2(3): 45-49
14. Egesie, U.G., Adelaiye, A.B., Ibu, J.O. & Egesie, O.J. (2006). Safety and hypoglycaemic properties of aqueous leaf extract of *Ocimum gratissimum* in streptozotocin induced diabetic rats. *Nigerian Journal of Physiological Sciences* 21(1-2): 31-35.
15. Lorke D. (1983).A new approach to practical acute toxicity testing. *Arch. Toxicol.*; 54: 275-287
16. Trease, G.E., & Evans, W.C. (1984). *Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine*. 13th Edition, Bailliere Tindall London.
17. Sofowora, L.A. (1984). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd, Ibadan. 85-82.
18. Vickers S, Duncan SA, Chen I-W, Rosegay A, Duggan DE (1990) *Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab Dispos* 18:138–145.
19. Jendrassik, L. & Grof, P. (1938): Determination of bilirubin in plasma. *Biochemistry* 297: 281.
20. Sherlock, S. & Lunec, J. (1951): Free radical and antioxidant system in health and disease. *British Journal of Hospital Medicine* 43: 334-344.
21. Forrester, R.L., Watagi, L.J., Silvermman, D.A. & Pierre, K.J. (1976): Enzymatic method for determination of carbon dioxide in serum. *Clinical Chemistry* 22: 243.36.
22. . Kolthoff I. M and Coetzee J. F. (1957). "Polarography in acetonitrile. I. Metal ions which have comparable polarographic properties in acetonitrile and in water." *J. Am. Chem. Soc.* 79 870-874.
23. Allain, C.C., Poor, L.S., Chan, C.S.G., Richmond, W. & Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry* 20: 470 – 475.
24. Edwards, C.R.W., Bouchier, I.A.D., Haslett, C. & Chilvers, E.E. (2008). Diabetes Mellitus in *Davidson's Principle and Practice of Medicine* (10th Edition) Churchill Livingstone, London. Pp 724 – 774.
25. Shahid, S.M. & Mahboob, T. (2008). Electrolytes and Na⁺ - K⁺ - ATPase: potential risk factors for the development of diabetic nephropathy. *Pakistan Journal of Pharmaceutical Sciences* 21(2): 172-179.
26. Ha, H., Hwang, I.A., Park, J.H. & Lee, H.B. (2008). Role of reactive oxygen species in thhe pathogenesis of diabetic nephropathy. *Diabetes Research & Clinical Practice* 82 suppl 1: s42-45.
27. Linda, S. C. (1998): Gastrointestinal system and endocrinology. Board review series physiology (3rd Edition). Lippincott Williams & Wilkins, Pp. 41-83.

589 28. Graw, A.R., Cowman, A., Reilly, D.S.O. & shepherd, J. (1995). *Clinical Biochemistry*.
590 New New York, Churchill,Livingstone. 87-90.

591 29. Eteng, M.U., Ibekwe, H.A., Essien, A.D., & Onyeama, H.P. (2008). Effects of
592 catharanthus rosens on Electrolyte Derangement induced by chlorpropamide (Diabenese)
593 on Normoglycemic Albino Wistar Rat. *Biology-Researchs* 6(2): 364-366.

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