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Comparative Evaluation of Diabetes Complication Using Liver Function Parameters as Indices between Male and Female Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

It is known that diabetes causes liver damage. On the other hand, because most cases of liver damage have been investigated in males, examining the relationship of this disease in both sexes is of great importance. This study was aimed at, comparatively evaluating diabetic complications

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using histopathological examination and liver function parameters as indices in alloxan-induced diabetic rats. A total of 24 male and female rats divided into four groups of six animals each were used for the experiment. Group A were female control, Group B were male control while group C and D were alloxan induced diabetic female and male rats respectively. Groups A and B were non diabetic rats fed with rat diet all through the experiment. Group C and D were diabetes induced with a single intraperitoneal injection of alloxan (120mg/kg). At the end of the inducement period, the rats were fasted overnight and sacrificed. Blood was collected by ocular puncture for the determination of fasting plasma glucose (FPG) and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total protein (TP) and albumin (ALB) using spectrophotometric method. Histology of the liver was determined by H and E technique. The results showed the experimental group C (365.83±43.6mg/dl) and D (368.94±43.93mg/dl) had significantly elevated levels of FPG when compared with the controls group A (116.00±9.11mg/dl) and B (108.33±5.5mg/dl) confirming the induction of diabetes (P=0.000). The liver of both male and female diabetic rats showed ballooning degeneration. In the males, these were arranged in normal sheets and cord around central vein in some areas of the center, on the other hand, the central vein of the female diabetic rats appears slightly enlarged containing blood clots and the arrangement of the hepatocytes in sheets or cords around the central vein was completely distorted. There was no significant difference (p>0.05) in the mean serum levels of ALP, AST, ALT, DB, TP and ALB in female diabetics when compared to male diabetic rats. The mean serum level of TB was significantly reduced (p=0.004) in female diabetics (0.53±0.05) than in the male diabetic albino rats (0.69±0.09). This study showed the liver histology and function are variably altered in diabetes mellitus. Further research on the causes of liver damage will help us to unravel the pathogenesis of diabetes and its complications.

Keywords: Diabetes mellitus; diabetic rats; liver function; alloxan; histopathological; bilirubin; alanine aminotransferase; aspartate aminotransferase; fatty liver; cell metabolism.

1. INTRODUCTION

Diabetes mellitus is a chronic condition caused by the body's failure to regulate glucose levels [1]. Diabetes is characterized by elevated blood glucose levels and hormonal modulation of glucose levels. Cellular resistance to insulin and a reduction in or absence of hormones that lower blood glucose are the causes of this unusual surge in glucose levels [2]. Almost every aspect of metabolism, including energy balance and the metabolism of glucose and lipids, is regulated in a sexually dimorphic way in males due to fundamental biological differences with females. This regulation affects the pathogenesis of most, if not all, diseases, including diabetes and obesity [3,4]. However, a lot of researchers usually use only male rodents in their studies. Because of the worry that the estrous cycle may cause variability in features that will confound experimental designs, females are not used as experimental subjects. It has been discovered that there is no greater variation in females than in males [5]. The fact that males show more dramatic disease phenotypes than females may potentially serve as a driving force behind the usage of male mice in the study of metabolic disease. For instance, research employing streptozotocin to generate insulin-deficient diabetes and studies using a high-fat diet to induce obesity both involve male subjects [3]. The effect of research findings in physiological studies is sometimes limited when they are restricted to a single sex, as the findings may only apply to half of the population. The National Institutes of Health (NIH) has lately required researchers to include both sexes in research designs in order to address this bias and include sex as a biological variable in preclinical research [6]. Being inclusive is not the only reason why studying male and female models is important. Instead, the comparison of the two sexes prompts inquiries that could not have been made otherwise, which could improve therapy.

Alloxan induces diabetes through a mechanism that essentially involves the beta cells of the pancreatic islets partially degrading and then producing less insulin overall, both in terms of quality and quantity [7]. It has been observed that alloxan-induced diabetes causes free radical production, which ultimately damages the pancreatic β-cells [8]. Some previous studies have also relied on alloxan to induce diabetes in experimental animals [9,10].

The liver is an essential organ in the human body that performs a variety of functions including

assisting with digestion, detoxification, immunity, metabolism, and vitamin storage [11]. Particularly exposed to reactive oxygen species, the liver can suffer damage from oxidative stress [12] and hyperglycemia has been shown to trigger oxidative stress [13].

The development of numerous other diabetic complications, including retinopathy, nephropathy, insulin resistance, and endothelial dysfunction, is subsequently accelerated up by chronic hyperglycemia in diabetes [14,15].

The panel test known as the liver function tests is frequently used to evaluate liver function. The tests that are frequently used in this regard include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total protein and albumin. The liver's hepatocellular functions are evaluated by the tests ALT, AST, and ALP, while its excretory functions are indicated by the tests TB and DB. There are modest concentrations of ALT in the heart and skeletal muscle, but much higher concentrations in the liver and kidney. All bodily tissues contain AST, however the liver, heart, skeletal muscle, and erythrocytes have the highest levels of activity. Serum increases of ALT activity are uncommon in disorders other than parenchymal liver diseases, despite the fact that serum levels of both AST and ALT are elevated whenever a disease process impairs the integrity of the liver cells [16]. ALT is a more liver-specific enzyme. Generally, ALT is located in the hepatocellular cytosol, whereas AST is located mostly within the mitochondria [17].

Alkaline phosphatase belongs to a family of zinc metalloenzymes that are found in the bile canaliculus's microvilli and a number of other tissues, including the placenta, intestines, and bone [18]. An elevation in bilirubin and ALP that is out of proportion to ALT and AST would indicate a cholestatic pattern [16]. Elevated levels of liver enzymes have been documented in diabetic population [19,20]. On the other hand, total protein and albumin assay are used for the assessment of the synthetic capacity of the liver.

It is imperative that both sexes be studied in pre-clinical investigations because there are undoubtedly differences between males and females in terms of sensitivity or responsiveness to medications and illnesses [21]. Moreover, considering sex- and gender-specific aspects is one of the first and simpler steps toward personalized and patient-centered care also in the management of Diabetes mellitus and its complications. Gender medicine analyzes the differences between men and women in human physiology, pathophysiology, and the clinical features of diseases, specifically evaluating the impact of sex as a biological and functional marker, and that of gender, which refers to a complex interrelation and integration of sex with psychological, social, ethnical and cultural behavior [22].

Although diabetes affects both sexes in individuals and in certain genetic animal models, most examinations of diabetic complications in fundamental science have focused on streptozotocin-induced diabetes in male mice only. National Institutes of Health now mandates that researchers include both sexes in their research [23]. Despite the extensive literature on diabetes, few exist that highlights similarities or differences in the progress of diabetic complications between the male and female gender. There is a noticeable gap in understanding how these biochemical changes manifest differently in male and female albino Wister rats. In the use of animal models, the female diabetic rats have been given minimal consideration. The lack of gender-specific insights hinders the development of tailored therapeutic strategies for managing diabetes-related complications. By systematically investigating and comparing the gender-specific variations in histopathology of liver and biochemical parameters in alloxan-induced diabetic rats, this research work has contributed to scientific knowledge on the pattern and progress of diabetes in both male and female diabetic rats, providing essential ground work for more nuanced and effective interventions in diabetes care. This study compared differences in histopathological alterations in tissues of the liver and biochemical indices of liver function in male and female alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of twenty-four males and female Wistar rats (*Rattus norvegicus*) weighing between 145g and 160g was purchased from the Research Aid Animal House, Federal University of Technology Owerri, Nigeria. The animals were kept in metallic cages and housed in a room with temperature of about 25°C. All experimental procedures were carried out in compliance with Guidelines for the Care and Use of Laboratory Animals.

2.2 Experimental Design and Animal Treatment

This study employed controlled experimental study design. The rats were acclimatized for two weeks before the experiment commenced and divided into four groups of six animals each. Diabetes was induced by a single intraperitonial (i.p) injection of alloxan (120mg/kg). The animals had free access to feed and water throughout the period of experiment and they were divided into four groups (group I-4) as follows:

Group A (normal control): This group was the nondiabetic female rats. They were fed with rat diet and water all through the period of experiment.
Group B (normal control): This group was the nondiabetic male rats. They were fed with rat diet and water all through the period of experiment.
Group C (diabetic female): These were the diabetic female rats. Diabetes was induced by a single intraperitonial(i.p) injection of alloxan(120mg/kg).
Group D (diabetic male): These were the diabetic male rats. Diabetes was induced by a single intraperitonial (i.p) injection of alloxan (120mg/kg).

The research animals were fasted and sacrificed after forty-eight (48 hours) of the alloxan-induction. Anesthesia was done using diethyl ether.

2.3 Collection of Blood Sample

At the end of the experimental feeding period, the rats were fasted overnight and sacrificed. Five milliliters (5ml) of whole blood was collected by ocular puncture into plain tubes, allowed to clot and the serum separated by centrifugation at 3500 rpm for five minutes. Samples were transported to De-life Family Diagnostic center, Owerri, for the analysis of biochemical parameters.

Collection of Organs: The liver tissues were collected at sacrifice and kept in ten percent buffered formalin and taken to the Histopathological unit of the Federal Medical Center Owerri for tissue processing.

2.4 Biochemical Analysis

Fasting plasma glucose was determined using the glucose oxidase peroxidase method described by Bergmeyer and Bernt [24]. Serum Alanine aminotransferase and Aspartate aminotransferase activity was determined using colorimetric method as described by Reitman and Frankel [25]. ALP activity was determined using the colorimetric method as described by King and Armstrong [26]. Bilirubin was concentration was determined using the colorimetric method as described by Jandrasssik and Grof [27]. Serum total protein (TP) was determined according to the spectrophotometric method described by Gornall et al. [28]. while Albumin (ALB) was determined by using the spectrophotometric method described by Doumas and Watson [29].

2.5 Histological Examination

The liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and then, following normal protocols, 5 µm thick slices were cut and stained with eosin and haematoxylin. A light microscope (Leica DM 1000 binocular microscope) was used to examine the slides, and 400× photomicrographs were taken.

2.6 Statistical Analysis

The statistical package for social sciences (SPSS) version 27 was used to evaluate data on all biochemical parameters. The Pearson correlation (r) was utilized to ascertain the link between the groups and the student t-test was employed to evaluate the difference in mean values between them. Statistical significance was defined as tests with a probability value of $p<0.05$.

3. RESULTS

The Histological section of liver of control rat seen in Fig. 1 showed no alteration.

Histopathological section of the liver of alloxan induced female diabetic rats seen in Fig. 2 showed increased vacuolation on the cytoplasm of hepatocytes (ballooning degeneration). The central vein appears slightly enlarged containing blood clot in some areas. The normal hepatocytes arrangement in sheets or cord around the central vein is completely distorted.

Fig. 3 shows the histopathological section of the liver of alloxan induced male diabetic rats. It showed hepatocytes undergoing ballooning degeneration (vacuolation of cytoplasm of hepatocytes) arranged in normal sheets and cord around central vein in some areas of the center.

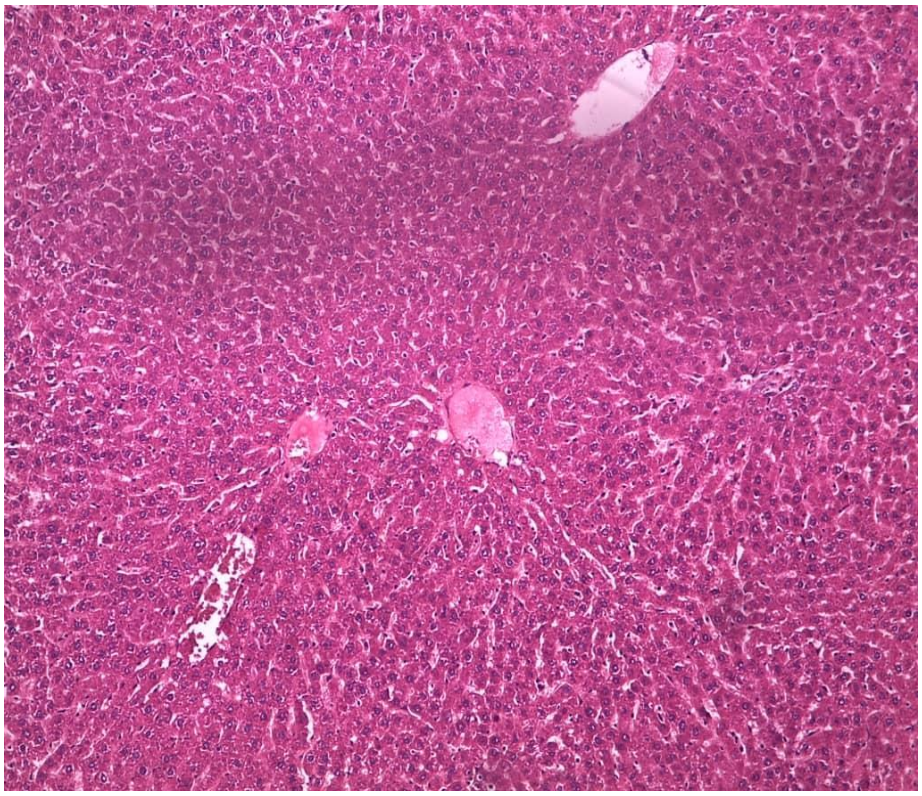

Fig. 1. Histological section of liver of control rat

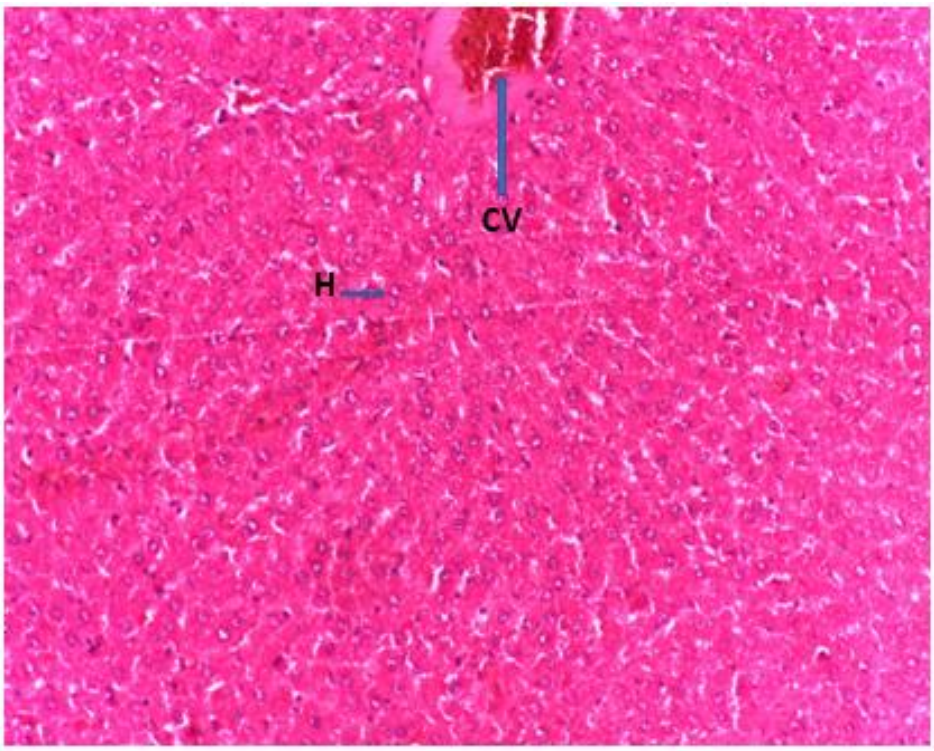

Fig. 2. Histopathological section of liver of female diabetic mice
Key: CV- Central vein
H- Hepatocytes

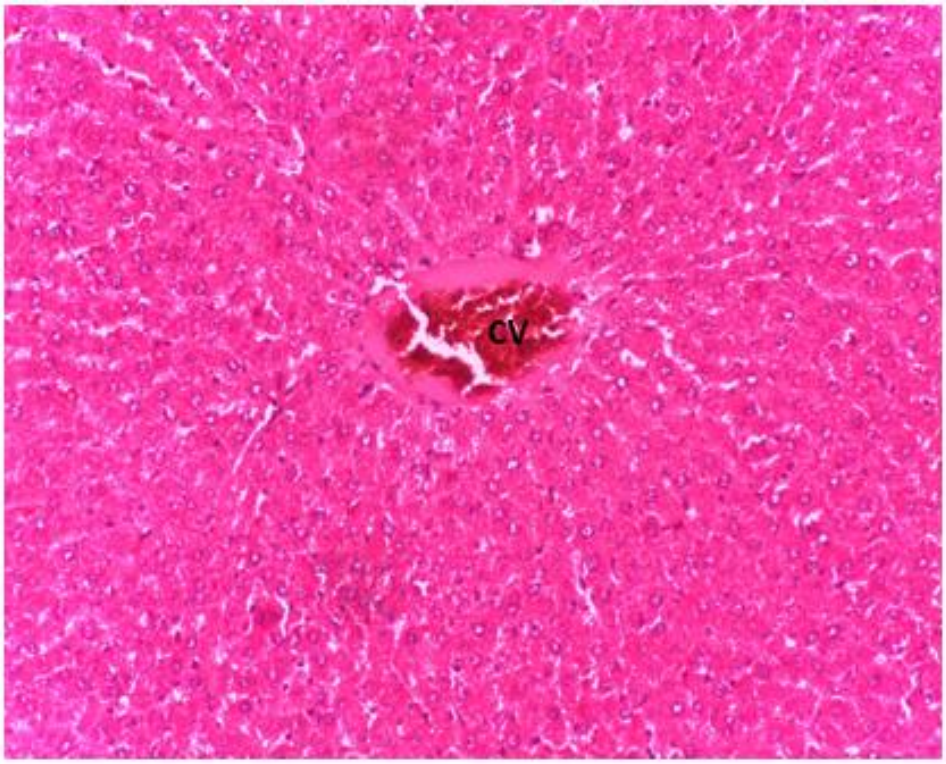

Fig. 3. Histopathological section of liver of male diabetic mice
Key: CV- Central vein
H- Hepatocytes

There was no significant difference (p=0.908) observed in the mean plasma glucose concentration in the female diabetic rats when compared to female control before induction. The mean value of plasma glucose level was significantly increased (p=0.000) in female diabetic rats when compared to female control after induction (See Table 1).

There was no significant difference (p=0.559) in the mean value of plasma glucose concentration observed in the male diabetic rats when compared to male control before induction. The mean value of plasma glucose level was significantly increased (p=0.000) in the male diabetic rats when compared to male control after induction (See Table 2).

Table 1. Levels fasting plasma glucose in female diabetic rats versus healthy female rats (control) before and After Inducement (Mean± SD)

Parameter	Period	Diabetic	Control	t-value	p-value
Glucose	Before Induction	113.17±9.24	113.67±4.68	0.12	0.908
	After Induction	365.83±43.61	108.33±5.54	14.35	0.000*

**Statistically significant at p<0.05.*

Table 2. Fasting plasma glucose levels in male diabetic rats versus healthy male rats (control) before and After Inducement (Mean± SD)

Parameter	Period	Diabetic	Control	t-value	p-value
Glucose	Before Induction	118.50±11.31	121.83±7.39	0.61	0.559
	After Induction	368.00±43.93	116.00±9.19	13.75	0.000*

**Statistically significant at p<0.05.*

There was no significant difference (p=0.704 and p=0.803) in the mean weight value of female diabetic rats when compared to female control before and after induction (See Table 3).

There was no significant difference (p=0.880 and p=0.989) in the mean weight value of male diabetic rats when compared to male control before and after induction (See Table 4).

There was no significant difference (p>0.05) observed in the mean serum levels of ALP, AST and ALT activities in female diabetics when compared to healthy female albino rats (Control). Also, there was no significant difference (p>0.05) observed in the mean serum levels of total bilirubin, conjugated bilirubin, total protein and albumin in female diabetics when compared to healthy female albino rats (Control). See Table 5.

There was no significant difference (p>0.05) observed in the mean serum activities of ALP, AST and ALT in male diabetics when compared to healthy male albino rats (Control). There was no significant difference (p>0.05) observed in the mean serum levels of total bilirubin, total protein and albumin in male diabetics when compared to healthy male albino rats (Control). However, the mean serum value of conjugated bilirubin was significantly reduced (p=0.030) in male diabetics when compared to healthy male albino rats (Control). (See Table 6).

There was no significant difference (p>0.05) observed in the mean serum activities of ALP, AST and ALT in the female diabetics when compared to male diabetic albino rats. Also, here was no significant difference (p>0.05) observed in the mean serum levels of conjugated bilirubin, total protein and albumin in the female diabetics when compared to male diabetic albino rats. On the other hand, the mean value of serum total bilirubin was significantly reduced (p=0.004) in the male diabetics when compared to o male diabetics albino rats (See Table 7).

Table 3. Mean weight levels in female diabetic rats versus healthy female rats (control) before and After Inducement (Mean± SD)

Parameter	Period	Diabetic	Control	t-value	p-value
Weight	Before Induction	149.17±10.40	149.17±10.21	0.39	0.704
	After Induction	155.50±10.24	157.16±10.25	0.26	0.803

**Statistically significant at p<0.05.*

Table 4. Mean Weight Levels in Male Diabetic Rats versus Healthy Male Rats (control) before and After Inducement (Mean± SD)

Parameter	Period	Diabetic	Control	t-value	p-value
Weight	Before Induction	144.67±9.71	145.67±12.44	0.16	0.880
	After Induction	149.17±10.40	149.17±10.21	0.10	0.989

**Statistically significant at p<0.05.*

Table 5. Serum Levels of ALP, AST, ALT, Total Bilirubin, Conjugated Bilirubin, Total Protein and Albumin in Female Diabetics Vs Female Control (Mean± SD)

Parameters	Female (Diabetics)	Female (Control)	t-value	p-value
ALP (IU/L)	590.58±208.26	449.71±62.81	1.59	0.144
AST (IU/L)	3.85±1.77	3.34±2.87	0.37	0.719
ALT (IU/L)	28.27±22.79	13.03±5.60		0.143
Total bilirubin (mg/dl)	0.53±0.05	0.63±0.14	1.58	0.115
Conjugated bilirubin (mg/dl)	0.19±0.08	0.34±0.21	1.72	0.164
Total Protein (mg/dl)	46.26±23.38	57.43±17.04	1.50	0.367
Albumin (mg/dl)	48.54±23.58	63.23±26.36	0.95	0.333

**Statistically significant at p<0.05.*

Table 6. Serum Levels of ALP, AST, ALT, total bilirubin, conjugated bilirubin, total protein and albumin in male diabetics Vs male control (Mean± SD)

Parameters	Male (Diabetics)	Male (Control)	t-value	p-value
ALP (IU/L)	897.70±641.77	913.44±293.12	0.06	0.958
AST (IU/L)	4.44±1.76	4.59±2.98	0.10	0.920
ALT (IU/L)	18.36±11.99	12.12±17.41	0.72	0.486
Total bilirubin (mg/dl)	0.69±0.09	0.74±0.07	1.00	0.341
Conjugated bilirubin (mg/dl)	0.25±0.04	0.43±0.17	2.53	0.030*
Total Protein (mg/dl)	58.16±16.49	57.76±16.10	0.043	0.967
Albumin (mg/dl)	44.66±18.19	63.04±19.35	1.60	0.140

**Statistically significant at p<0.05.*

Table 7. Serum Levels of ALP, AST, ALT, total bilirubin, conjugated bilirubin, total protein and albumin in female diabetics Vs male diabetics (Mean± SD)

Parameters	Female (Diabetics)	Male (Diabetics)	t-value	p-value
ALP (IU/L)	590.58±208.26	897.70±641.77	0.06	0.291
AST (IU/L)	3.85±1.77	4.44±1.76	0.10	0.578
ALT (IU/L)	28.27±22.79	18.36±11.99	0.72	0.369
Total bilirubin (mg/dl)	0.53±0.05	0.69±0.09	1.00	0.004
Conjugated bilirubin (mg/dl)	0.19±0.08	0.25±0.04	2.53	0.197
Total Protein (mg/dl)	46.26±23.38	58.16±16.49	0.043	0.332
Albumin (mg/dl)	48.54±23.58	44.66±18.19	1.60	0.756

**Statistically significant at p<0.05.*

There was a no significant negative correlation of glucose concentration with ALP (r=-0.33, p=0.517), AST (r=-0.39, p=0.439), ALT (r=-0.57, p=0.240), total bilirubin (r=-0.44, p=0.383), conjugated bilirubin (r=-0.33, p=0.442), total protein (r=-0.44, p=0.381) and albumin (r=-0.01, p=0.986) in female diabetic albino rats (See Table 8).

There was a no significant negative correlation of glucose concentration with ALP (r=-0.24, p=0.641), AST (r=-0.61, p=0.196) in male diabetic albino rats. There was a no significant positive correlation of glucose concentration with AST (r=-0.11, p=0.835), ALT (r=0.11, p=0.835), total bilirubin (r=0.19, p=0.708), conjugated bilirubin (r=0.51, p=0.306), total protein (r=-0.54, p=0.268) and albumin (r=-0.38, p=0.460) in male diabetic albino rats (See Table 9).

4. DISCUSSION

The liver is one of the major organs affected by diabetes mellitus. The impact of gender on diabetes related metabolic changes in animal models are rarely reported. Most literatures highlight majorly the effect of therapeutic interventions on diabetes induced rats without comparatively x-raying the impact of gender on these metabolic changes [30,31].

The results of this present work, elucidates the differences in histopathological tissues of liver and liver function parameters between male and female diabetic rats.

Numerous results have validated that alloxan is capable of inducing diabetes in animal models [30-32], the result of this work affirms this assertion. Blood glucose levels in both diabetic male and female rats were statistically elevated compared to the control rats. This may be because there is quick absorption of alloxan by pancreatic beta cells, which has been suggested to be one of the key factors determining alloxan diabetogenicity [33].

Table 8. Correlation of Glucose Concentration with Liver Function Parameters in Female Diabetics

Dependent Variable	N	R	p-value
ALP	6	-0.33	0.517
AST	6	-0.39	0.439
ALT	6	-0.57	0.240
Total bilirubin	6	-0.44	0.383
Conjugated bilirubin	6	-0.33	0.442
Total protein	6	-0.44	0.381
Albumin	6	-0.01	0.986

**Statistically significant at p<0.05.*

Table 9. Correlation of Glucose Concentration with Liver Function Parameters in Male Diabetics

Dependent Variable	N	R	p-value
ALP	6	-0.24	0.641
AST	6	-0.61	0.196
ALT	6	0.11	0.835
Total bilirubin	6	0.19	0.708
Conjugated bilirubin	6	0.51	0.306
Total protein	6	0.54	0.268
Albumin	6	0.38	0.460

**Statistically significant at p<0.05.*

However, there was no statistical difference between the blood glucose level of male diabetic rats compared to female diabetic rats. This may mean that regulation of plasma glucose in both male and female diabetics follow the same pattern and as such hyperglycemia affects both gender in a similar manner.

In this study, the mean body weights of the diabetic rats were not significantly different from the normal control. This is not consistent with the results of previous studies which showed a significant decrease in body weight of alloxan induced diabetic rats [34]. It is also in contrast to Hassan *et al.* who reported an increase in the mean body weights of diabetic rats [35].

On the other hand, the comparative evaluation of liver function in male and female alloxan-induced diabetic rats revealed that there was no significant difference in the mean values of ALP, AST, ALT, conjugated bilirubin, total protein and albumin in female diabetics when compared to male diabetic rats. Only the mean value of total bilirubin was significantly reduced in female diabetics when compared to male diabetic albino rats. This may be a short term indication that the excretory function of the liver may be impaired more in male diabetics relative to the female diabetics. Serum AST, ALT and ALP, are the enzyme biomarkers to monitor the liver structural integrity and damage and aids in the clinical diagnosis of liver toxicity conditions. The liver serves as a center for the metabolism of nutrients and the excretion of waste metabolites.

However, the histopathological examination of the liver tissues, revealed increased vacuolation on the cytoplasm of hepatocytes of both male and female diabetic rats. This is because the liver is one of the primary organs vulnerable to the effects of hyperglycemia-induced oxidative stress, which may result in liver tissue damage [36]. The liver is a collection of insulin-sensitive tissues. More so, the normal hepatocytes arrangement in sheets or cord around the central vein appeared distorted in the diabetic female rats. These histopathological alterations may be the reason for the non-significant increase in serum concentrations of AST, ALT and ALP of the female diabetic rats compared to the control group, suggesting that the liver may have just began undergoing structural compromise. The non-significant reduction in total protein and albumin may imply the beginning of a progressive decline in the liver synthetic function in the female diabetic rats. The histopathological changes in the male diabetic rats were milder. Similar to this work, Gamde *et al*. had also observed inflammation and apoptotic cells on liver of diabetic rats [34]. Also, some other studies suggested that male diabetic rats are

more prone to hepatic steatosis, while female rats exhibit a higher prevalence of liver fibrosis. These differences are attributed to variations in metabolic rates, hormone levels, and susceptibility to oxidative stress [3]. Furthermore, Luchessi *et al*. had queried if the observed histopathological liver injuries were the result of toxic effects of alloxan or streptozotocin or linked to the drug-induced diabetic hyperglycemic state, the result of their work revealed that untreated diabetic rats showed liver morphological changes characterized by hepatic sinusoidal enlargement and micro- and macrovesicular hepatocyte fatty degeneration with progressive liver structure loss, steatohepatitis, and periportal fibrosis [37].

In the work of Sohaib *et al*., the serum levels of the conjugated bilirubin in control male rats (0.24±0.02) were similar to that of the control male rats (0.25±00) in this study [32]. However, Sohaib *et al*. used only male rats for their experiment. Diabetic male rats (0.16±0.03) were lower in value than diabetic male rats (0.43±0.2) in this work [32].

5. CONCLUSION

This study revealed moderate alterations in the histology of the male and female diabetic rats as well as in the serum bilirubin levels which may hamper the excretory function of the liver. Further research on the causes of liver damage will help us to unravel the pathogenesis of diabetes and its complications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ogbodo EC, Okafor CC, Ogah HGO, Ezeugwunne IP, Igwebuobi CF, Okezie AO, Agada UN, Amah AK, Odumodu IO. Thyroid hormone profiling and enzymatic antioxidant status in diagnosis and management of type-ii-diabetes mellitus: a review of literature. World J Pharmaceut Life Sci. 2019;5(12):6-21.
2. Ogbu ISI, Ejike-Odeh EJ, Obeagu EI. Insulin Resistance: A Review. Elite J Lab Med. 2024; 2(3):11-28.
3. Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. Biol Sex Differ. 2015; 6:14. DOI: 10.1186/s13293-015-0033-y.
4. Mauvais-Jarvis F, Arnold AP, Reue K. A Guide for the Design of Pre-clinical Studies on Sex Differences in Metabolism. Cell Metabolism. 2017; 25(6): 1216-1230. DOI: 10.1016/J.CMET.2017.04.033.
5. Becker JB, Prendergast BJ, Liang JW. Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. Biol Sex Differ. 2016; 7:34.
6. Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. Nature. 2014; 509(7500):282-283. DOI: 10.1038/509282a.
7. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas). 2017;53(6):365-374.
8. Martin O, Blessing OO, Gloria OO. Investigation of the Biochemical Indices Related to Differential Response of Fasting Blood Glucose to Alloxan-Induced Diabetic Rats. Int J Basic Sci Technol. 2023; 9(1): 26-33. Doi: 10.5555/Colb2178.
9. Analike RA, Ezeugwunne IP, Ogbuebuna UE, Ogbodo EC, Oguaka VN, Amah AK, Ahaneku JE, Ahaneku GI. Effect of Sida corymbosa leaf extract on the activity of serum liver enzymes of alloxan-induced diabetic albino wistar rats in College of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. Acta Medica Scientia. 2018; 5(2): 5-7.
10. Ogbodo EC, Emeka NC, Ezeugwunne IP, Analike RA, DiKe CC, Njoku MC, Oguaka VN, Amah UK, Okwara EC, Asebioyo SJ. Effect of Ethanoic Leaf Extract of

Phyllantus Amarus On Fasting Blood Glucose, Bilirubin, Albumin and Total Protein On Diabetic Induced Albino Wistar Rats in College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. Global Scientific J. 2017; 5(7): 78-85.

11. Kalra A, Yetiskul E, Wehrle CJ, Tuma F. Physiology, Liver. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available:https://www.ncbi.nlm.nih.gov/books/NBK535438/
12. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative Stress in Liver Pathophysiology and Disease. Antioxidants (Basel). 2023; 12(9):1653. DOI: 10.3390/antiox12091653.
13. Binjawhar DN, Alhazmi AT, Jawhar WNB, Saeed WM, Safi SZ. Hyperglycemia-induced oxidative stress and epigenetic regulation of ET-1 gene in endothelial cells. Front. Genet. 2023; 14. DOI: 10.3389/fgene.2023.1167773.
14. Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail IS. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. Biomed Res Int. 2014; 2014:801269. DOI: 10.1155/2014/801269.
15. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271-281. DOI: 10.1016/j.diabres.2018.02.023.
16. Lala V, Zubair M, Minter DA. Liver Function Tests. [Updated 2023 Jul 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available:https://www.ncbi.nlm.nih.gov/books/NBK482489/
17. Mandal A, Bhattarai B, Kafle P, Khalid M, Jonnadula SK, Lamicchane J, Kanth R, Gayam V. Elevated Liver Enzymes in Patients with Type 2 Diabetes Mellitus and Non-alcoholic Fatty Liver Disease. Cureus. 2018 Nov 23;10(11):e3626. DOI: 10.7759/cureus.3626.
18. Iluz-Freundlich D, Zhang M, Uhanova J, Minuk GY. The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. Ann Hepatol. 2020; 19(2):204-208.
19. Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N. Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinol Diabetes Metab. 2020; 3(2): e00116. DOI: 10.1002/edm2.116.
20. Noroozi KM, Khalili P, Ayoobi F, Esmaeili-Nadimi A, La Vecchia C, Jamali Z. Serum liver enzymes and diabetes from the Rafsanjan cohort study. BMC Endocr Disord. 2022; 22: 127. Available:https://doi.org/10.1186/s12902-022-01042-2.
21. Liu S, Mauvais-Jarvis F. Minireview: Estrogenic Protection of Beta-cell Failure in Metabolic Diseases. Endocrinol. 2010; 151:859–864.
22. Machluf Y, Chaiter Y, Tal O. Gender medicine: Lessons from COVID-19 and other medical conditions for designing health policy. World J Clin Cases. 2020 Sep 6;8(17):3645-3668. DOI: 10.12998/wjcc.v8.i17.3645.
23. Negera GZ, Weldegebriel B, Fekadu G. Acute Complications of Diabetes and its Predictors among Adult Diabetic Patients at Jimma Medical Center, Southwest Ethiopia. Diabetes Metab Syndr Obes. 2020; 13:1237-1242. DOI: 10.2147/DMSO.S249163.
24. Bergmeyer HU, Bernt E. Determination of glucose with oxidase and peroxidase, In Bergmeyer HU (ed), Methods of enzymatic analysis. Verlag Chemie/Academic Press, New York, NY. 1974;1205–1215. Available:https://cir.nii.ac.jp/crid/1571417125528254848, on 22 November 2023.
25. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American J Clin Pathol. 1957; 28:56-63.
26. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activities. Canadian Med Assoc J. 1943; 31:376-381.
27. Jandrasssik, L. and Grof, P. Bilirubin estimation. Biochem. 1938; 297:81-84.
28. Gornall AG, Berdawill CJ, David MM. Determination of seruml proteins by means of the biuret method. J Biol Chem. 1949; 177:751.
29. Doumas B, Watson, W. The determination of serum albumin using Bromocresol Green (BCG). Clin. Chimica Acta. 1971;31:87-89.

30. Ezeugwunne IP, Umeaka BK, Ogbodo EC, Okpogba AN, Analike RA, Okwara JE, Onyegbule OA, Okwara EC. Effect of Phyllantus Amarus Leaf Extract on the Serum Liver Enzymes of Alloxan-Induced Diabetic Albino Wistar Rats in College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. European J Biomed Pharmaceut Sci. 2018; 5(2): 61-66.
31. Ogbodo EC, Onyekwelu PC, Ezeugwunne IP, Madukwe DUP, Analike RA, Okeke KU, Njoku CM, Oguaka VN, Okwara EC, Amah UK. Effect of Sida corymbosa leaf extract on the serum blood glucose, total protein, albumin and bilirubin levels of alloxan-induced diabetic albino wistar rats in College of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. Int J Herbs Pharmacol Res. 2017; 6(2): 37-42.
32. Sohaib M, Khan AU, Rabbani. Antidiabetic, Antioxidative and anti-hyperlipidemic Effects of Strawberry fruit extract in alloxan-induced diabetic Rats. Foods. 2023; 12(15): 2911.
33. Lal S, Kumar V, Sharma SK, Priyadarshi A, Sardana M. Diabetogenic potential of Alloxan in Albino Mice. IOSR J Biotechnol Biochem. 2020; 6(4): 48-55.
DOI: 10.9790/264X-0604014855.
34. Gamde SM, Ugwah-Oguejiofor CJ, Garba A, Avwioro GO, Moronkeji A, Jimoh AA. Histologic and Biochemical Effect of Balanite aegyptiaca Fruit Extract on Alloxan-Induced Diabetes in Wistar Rats. Ethiop J Health Sci. 2023; 33(3):441-450.
DOI: 10.4314/ejhs.v33i3.7.
35. Hassan M D, Abdulkarim ZI, Mary M. Effects of Balanites aegyptiaca fruit pericarp on fructose-induced hyperglycemia and hyperlipidemia in rats. Asian J Biotechnol. 2020; 12:87–96.
36. Bae CS, Lee Y, Ahn T. Therapeutic treatments for diabetes mellitus-induced liver injury by regulating oxidative stress and inflammation. Appl Microsc. 2023; 53(1):4.
DOI: 10.1186/s42649-023-00089-2.
37. Lucchesi AN, Cassettari LL, Spadella CT. Alloxan-induced diabetes causes morphological and ultrastructural changes in rat liver that resemble the natural history of chronic fatty liver disease in humans. J Diabetes Res. 2015; 2015:494578.
DOI: 10.1155/2015/494578.

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