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Evaluation of Biochemical Changes in Induced Diabetic Male Rats by Different Methods.

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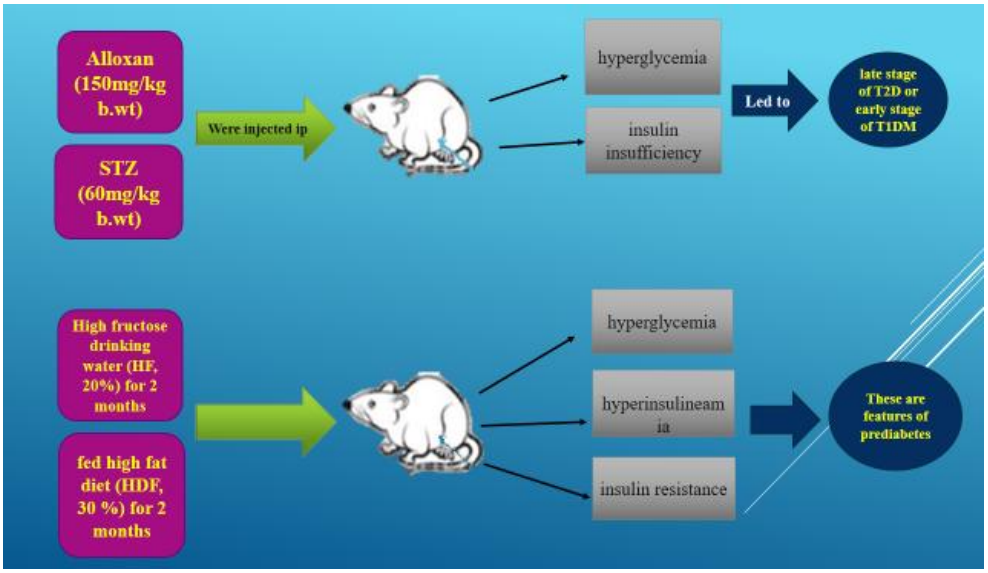
ABSTRACT

In Egypt, according to International Diabetes Federation (IDF), 10.9 million Egyptians were suffering from diabetes in 2021. Different models are used to induce diabetes in different animals. This study aimed to evaluate the biochemical changes due to induction of diabetes in rats with different methods. Methods: male albino rats were used in this study. First group is the control group was injected with equivalent amount of 0.9% saline ip to that used for alloxan and STZ preparation, second group was injected with150 mg alloxan/ kg b.wt, third group injected with Streptozotocin (STZ) 60mg/kg b.w. The fourth group drank high fructose (HF, 20% solution) for 2 months and the fifth group fed high fat diet (HDF, 30 %) for 2 months. Results: Both alloxan and STZ induced T1DM which was characterized by very high elevation in the blood glucose (405.20±20.28and 424.40±10.31mg/dl) in both groups respectively vs control group (86.70±1.45mg/dl). Also, high decreases of insulin level were recorded in the same groups. HF and HFD groups revealed the presence of slightly hyperglycemia in these rats. Both blood glucose (104.30±3.28 and 104.20±3.48mg/dl) respectively and insulin levels increased in both groups. Many changes of both liver and kidney functions tests altered. All groups had increased oxidative stress marker (MDA) which was associated with decreased antioxidants markers. Conclusion: the used models for diabetes induction can be used for assessment the diabetes complications and treatment trials.

Graphical abstract



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Introduction

According to the International Diabetes Federation (IDF, 2021), 537 million people are estimated to be living with diabetes, representing 9.8% of the global adult population (20–79 years). This number is expected to increase to 643 million (10.8%) in 2030 and 783 million (11.2%) in 2045. In Egypt, according to IDF, 10.9 million Egyptians were suffering from diabetes in 2021 [1]. Diabetes Mellitus (DM) is a group of autoimmune, metabolic and genetic disease can cause due to drugs, surgery, infections, decrease physical activity and obesity. DM is characterized by disorders in protein, fat and carbohydrate metabolism which accompanied by hyperglycemia that caused by dysfunction in insulin action, secretion or both [2, 3].

There are three types of diabetes, type1 known as T1DM, type2 known as T2DM and gestational DM (GDM). T1DM is a chronic autoimmune disorder characterized by deficiency in insulin secretion due to the destruction of β-cells in the pancreas by the cellular autoimmune process [2]. T2DM is characterized by insulin resistance (IR) happens due to dysfunction of different cellular pathways, which leads to decrease the response or sensitivity of cells towards insulin [4]. Serum insulin concentrations may be significantly higher in T2DM [5]. It has been proposed that this hyperinsulinemia is the result of both increased insulin production and decreased insulin clearance [6]. Gestational DM is an impairment in glucose that is recognized during pregnancy [7].

The symptoms of diabetes including polyuria, polydipsia, polyphagia, and blurry vision. Sometimes symptoms are mild or absent for many years among people who haveT2DM especially when hyperglycemia is mild. There are also many complications of diabetes which common between T1DM and T2DM patients. At the same time, these complications lead to morbidity and many deaths [8]. Oxidative stress (OS) caused by imbalance between removal and formation of free radicals. OS is generate a high amount of reactive oxygen species (ROS) and prevents the antioxidant defense from destroying ROS, causing many diseases including diabetes [9] and insulin resistance [10]. Frequently scientists used rats and mice from the rodent group to replicate phenotype and pathogenesis disease as human. These animal models are used to study biochemical changes as well as the complications of diabetes and the possible treatments for it. Alloxan, streptozotocin (STZ), high fructose (HF) in water or diet and high fat diet (HFD) are usually used to induce diabetes.

Alloxan exerts its cytotoxic effects by generating ROS within pancreatic β-cells, causing OS and destruction of insulin-producing cells. STZ enters β- cells via the glucose transporter (GLUT2) and induces DNA alkylation, thereby triggering DNA strand breaks and cell death. These distinct mechanisms of action highlight the specificity of alloxan and STZ for β-cells, making them valuable tools in the creation of diabetic animal models for studying the biochemical changes associated with DM. [11]

Therefore, the present work aimed to evaluate the biochemical and OS changes that may happen in male albino rats after induction of diabetes by these different methods for eight weeks.

1. Material and methods

Animals

Total 50 adult male Wistar albino rats (body weight 150-180 g) were obtained from VACSERA Company, Cairo, Egypt. Rats were stayed at the animal facility in the medical Physiology lab, Faculty of Medicine, Al-Azhar University (boys), Cairo, Egypt. They were allowed to adapt for two weeks before starting the experiment. Rats were kept under standard condition in the lab, exposed to (12/12h) light/dark cycle and a temperature 25 ± 2 °C. Animals were housed in cages (5 rats / cage) with free access to standard laboratory chow diet from (ALSALAH Feed Company, Cairo, Egypt) and water *ad libitum*. The study was conducted whereby the international guidelines for animal experiment and consent by Ethical Committee of the Medical Physiology Lab, Faculty of medicine, Al-Azhar University (boys), Cairo, Egypt.

Induction of Diabetes

1-Alloxan

Alloxan was obtained from (Sigma pharma chemicals company, Cairo, Egypt). It was prepared by dissolving 150mg/kg body weight (b. wt) in 0.9% NaCl. To induce diabetes by alloxan, rats were fasted overnight and injected with a single dose of alloxan intraperitoneally (ip) immediately after preparation [12].

2-Streptozotocin (STZ)

Streptozotocin (s 0130-1G) was obtained from Sigma (IG TECHNOLOGY, researcher supplier Company, Cairo, Egypt) and stored at -20°C to avoid degradation. STZ was freshly prepared by dissolving (60 mg/kg b.wt) in saline. To induce diabetes by STZ, rats were fasted overnight and injected by a single dose of STZ ip [13]. Progression of diabetes was confirmed in blood by assessment of the blood glucose level using glucometer, three days after alloxan and STZ injection. Rats with blood glucose levels equal or higher than 200 mg/dl were treated as diabetic [14].

3- High Fructose (HF) drinking water

Fructose was obtained from (Rich Diet Company, Cairo, Egypt). Rats in fructose group were subjected to fructose (20%) in fresh drinking water daily for 8 weeks [15].

4- High Fat diet (HFD)

Butter was used as a HFD and was obtained from a local market. Rats in HFD group were fed fat (30%) in their daily diet for 8 weeks. Normal Diet (ND) with the following macronutrient composition: 4.6 % fat, 23 % protein and 72.4 Carbohydrates. The butter was added to normal diet so the composition of HFD became 30 % fat, 23 % protein and 47 % carbohydrates [16].

Experimental design

In this study, animals were randomly divided into 5 groups; each group had 10 adult male rats:

1- Control groups (non- diabetic rats).
2- Alloxan group.
3- STZ group.

4- HF drinking water group.
5- HFD group.

Blood sampling

At the end of eight weeks, rats were fasted overnight for 12 h, with free drinking water. Blood was collected from retro-orbital venous plexus of the eye of each rat by using heparinized capillary tube. Blood was collected into two dry clean glass tubes. The first tube had EDTA to measure glycosylated hemoglobin (HbA1c), the second tube had normal blood and let it clot to get serum. To obtain the serum, blood samples were centrifuged at 3000 RPM for 10 min. The supernatant sera were collected into Eppendorf tubes and stored at -20°C for biochemical analysis.

Biochemical parameters

Serum fasting blood glucose (FBG) [17], serum insulin level [18], HbA1c [19], aspartate aminotransferase (AST), alanine aminotransferase (ALT) [20], alkaline phosphate (ALP) [21], blood urea [22], creatinine [23], uric acid (UA) [24], malondialdehyde (MDA) [25], glutathione (GSH) [26], superoxide dismutase (SOD) [27], catalase (CAT) [28] and glutathione peroxidase (Gpx) [29] were measured in sera.

Homa IR was calculated using the following equation HOMA-IR = [glucose (mg/dL) × insulin (μIU/L)] / 405 [30].

Statistical Analysis

Analysis of data was measured using computer program SPSS. The results are expressed as the mean ± standard error (M± SE). The mean values of different groups were compared using T- Test and a one-way analysis of variance (ANOVA).

2. Results

Body weight: at the beginning of the experiment, there were no differences in the initial body weights means between different groups. At the end of the experiment, the reduction of weight in alloxan and STZ groups were highly significant compared to their initial weights and final weights of control group. At the end of the experiment, the elevation of final body weights in HF and HFD groups were highly significant compared to their initial weights and the final weights of control group (Table 1).

Table (1): Initial and final body weights (g) of control and different treated groups.

Groups / Parameters	control	Alloxan	STZ	HF	HFD
Initial body weight	167.70 ±11.59	165.60 ±9.13	168.70 ±10.29	168.20 ±9.00	168.89±10.16
Final body weight	199.50 ±12.32[b]	134.40 ±6.99[ab]	136.5±8.52 [ab]	242.2±13.97 [ab]	264.80±14.54 [ab]

Means ±SE of 10 animals.
a = P<0.05 when treated groups compared with control group.
b = P<0.05 when each initial weight compared to the final weight of the same group.

Table (2): Blood glucose, insulin level, Homa-IR and HbA1C in control and different treated groups.

Groups / Parameters	Control	Alloxan	STZ	HF	HFD
FBG (mg/dl)	86.70±1.45	405.20±20.28**	424.40±10.31**	104.30±3.28*	104.20±3.48*
%Change from control		367.36	389.50	20.30	20.18
Insulin level (μIU/L)	5.48±0.24	0.79±0.067**	0.78±0.052**	7.86±0.15*	7.86±0.089*
%Change from control		-85.58	-85.77	43.49	43.43
Homa-IR	1.17±0.05	0.78±0.068*	0.82±0.06*	2.02±0.078**	2.02±.072**
%Change from control		-33.16	-29.31	73.18	73.01
HbA1C (%)	2.96±0.46	8.43±0.61**	8.51±0.86**	3.08±0.33	4.09±0.85*
%Change from control		184.78	187.5	4.05	38.18

All results are Means ±SE of 10 animals.
* = P<0.05 and ** = P<0.01 as compared to control group.

As shown in Table (2), Alloxan and STZ groups showed highly significant increases in FBG and HbA1c and highly significant decreases in serum insulin levels and Homa-IR (P< 0.01). HF and HFD groups showed significant increases in FBG level (P< 0.05) and highly significant increases in serum insulin and Homa-IR (P< 0.01). HbA1c (P< 0.05) was significantly elevated in HFD group but was showed non-significant change in HF group compared to control group.

Percentage Change from control = $\frac{X\ treated\ group - X\ control}{X\ control} \times 100$

Liver enzymes: alloxan and STZ groups showed highly significant increases in ALT (P<0.01) but significant increases AST and ALP in the same groups (P<0.05) compared to control group. HFD group had highly significant increases in ALT, AST and ALP (P<0.01) compared to control group. HF group showed non-significant changes in liver enzymes compared to control group (Table 3).

Table (3): ALT, AST and ALP activities in control and different treated groups.

Groups / Parameters	control	Alloxan	STZ	HF	HFD
AST (U/L)	95.00±2.48	140.10±4.03*	141.00±5.51*	87.40±4.75	168.70±8.14**
%change from control		47.47	48.42	-8	77.58
ALT (U/L)	35.00±1.04	82.30±2.52**	83.90±1.95**	37.30±1.21	69.40±4.04**
%change from control		135.14	139.71	6.57	98.29
ALP (U/L)	66.20±1.84	88.10±1.97*	96.90±5.57*	72.10±5.47	117.90±6.48**
%change from control		33.08	46.37	8.91	78.10

All results are Means ±SE of 10 animals.
* = P<0.05 and ** = P<0.01 as compared to control group.

Kidney functions: blood urea was elevated significantly in alloxan, HF and HFD groups (P<0.05). STZ group had no significant change in blood urea compared to control group. All groups showed no significant changes in serum creatinine levels. Alloxan, STZ and HFD showed highly significant increase in uric acid (P<0.01). HF group showed non-significant change in uric acid compared to control group (Table 4).

Table (4): Blood Urea, creatinine and uric acid in control and different treated groups.

Groups / Parameters	control	Alloxan	STZ	HF	HFD
Urea (mg/dL)	17.00±0.63	20.20±1.31*	18.40±0.65	23.20±1.55*	21.00±1.26*
%change from control		18.82	8.24	36.47	23.53
Creatinine(mg/dL)	0.55±0.12	0.60±0.03	0.64±0.05	0.60±0.05	0.57±0.05
%change from control		9.09	16.36	9.09	3.64
UA (mg/dL)	1.58±0.13	2.64±0.14**	2.77±0.09**	1.39±0.14	2.68±0.19**
%change from control		67.09	75.32	-12.03	69.62

All results are means ±SE of 10 animals.
* = P<0.05 and ** = P<0.01 as compared to control group.

As shown in Table (5), Alloxan and STZ groups showed highly significant elevation in serum MDA levels compared to control rats (P<0.01), but HFD group showed significant increase in MDA levels (P<0.05). CAT was reduced significantly in alloxan and STZ groups (P<0.05). Furthermore non-significantly change in HFD groups. Alloxan and STZ groups showed a non-significantly changes in SOD levels but was elevated significantly in HFD group (P<0.05). GSH was significantly reduced in alloxan and STZ groups (P<0.05). HFD showed non-significant change in GSH. Gpx was significantly reduced in alloxan, STZ and HFD groups (P<0.05). HF group showed non- significant change in MDA, GSH, CAT and Gpx levels compared to control group.

Table (5): Oxidative stress marker (MDA), glutathione (GSH) and antioxidant activities (SOD, catalase and Gpx).

	Control	Alloxan	STZ	HF	HFD
Serum MDA (µmol/L)	5.50±0.36	10.55±0.62**	10.16±0.44**	5.15±0.33	7.70±0.41*
%change from control		91.82	84.73	-6.36	40.00
GSH (µmol/L)	5.03±0.18	3.82±0.14*	3.89±0.26*	5.18±0.37	5.88±0.47
%change from control		-24.06	-22.66	2.98	16.90
SOD (mmol/L)	115.70±2.98	108.10±4.35	111.30±5.25	111.30±6.04	72.60±3.92*
%change from control		-6.57	-3.80	-3.80	-37.25
Catalase (U/mL)	27.60±1.25	22.70±0.99*	23.70±1.05*	26.10±1.52	24.30±2.01
%change from control		-17.75	-14.13	-5.43	-11.96
Gpx(U/mL)	26.14±1.39	16.13±1.17*	15.55±1.01*	25.56±2.90	20.07±1.15*
%change from control		-38.29	-40.51	-2.22	-23.22

All results are means ±SE of 10 animals.
* = P<0.05 and ** = P<0.01 as compared to control group.

3. Discussion

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia [31]. Hyperglycemia activates many metabolic or signaling pathways that not only attempt to dispose excessive glucose but also generate more ROS, leading to OS and β-cell failure and cell death [32]. This work was conducted to evaluate the biochemical and OS changes in induced diabetic male rats by different methods. Four methods were used Alloxan, STZ, 20% HF drinking water and 30% HFD. High-dose STZ leads to impair insulin secretion and T1DM, but low-dose STZ leads to induce a moderate impairment of

insulin secretion which is a feature of the actual later stage of T2DM [11]. The dose of alloxan is necessary for inducing different types of diabetes. High doses of alloxan can lead to insulin deficiency as well as T1DM but low doses of alloxan can induce insulin resistance [33].

In this study, the reduction of body weight in alloxan and STZ groups at the end of the experiment were highly significant compared to their initial weights and the final weights of control group. This finding was supported by Ul Haq *et al* [34] and Samadi-Noshahr *et al* [35] who found a significant decrease in body weight in wistar male rats after the injection of alloxan and STZ respectively. These results might be due to increase the wasting muscles and reduce the protein and fatty acid of muscles and lipid tissues, and this may lead to muscle and fat catabolism and dehydration [36].At the end of experiment, the elevation of final body weights in HF and HFD groups were highly significant compared to their initial weights and the final weights of control group. These findings are in accordance with Marques *et al* [37] and Moreno-Fernández *et al* [10] who found a significant increase in body weight in wistar male rats after feeding by HF and HFD. These results might be due to imbalance between energy uptake and energy expenditure which lead to accumulation of the fat in white adipose tissue [10]. Fasting blood glucose is directly proportional to the severity of the DM. The American Diabetes Association (ADA) recommends using HbA1c for the diagnosis of pre-diabetes [38]. It has been shown that Homa-IR has a strong connection with the insulin tolerance in rats, and it can be used as a good indicator of insulin resistance in rats [39].

In this study, T1DM was induced by an ip injection of alloxan (150mg/kg b.wt). The present results demonstrated increases in FBG levels and HbA1c and decreases in insulin level and Homa-IR. These finding was supported by the study of Mistry *et al* [40] who found insufficient production of insulin by β-cells of islets of Langerhans in the pancreas after the induction of alloxan. This hyperglycemia could be a result of alloxan-induced ROS, in addition to a simultaneous huge elevation in cytosolic calcium concentricity that led to a rapid damage of islet cells of pancreas and a reduction in release or synthesis of insulin in these rats. In the present study, T1DM was detected in rats by an ip injection of STZ (60 mg/kg b. wt). The symptoms were characterized by high significant increases in FBG, HbA1c and high significant decreases in serum insulin and Homa-IR. These might be due to the toxic effect of STZ on pancreatic β-cells. These results are in accordance with Peña-Montes *et al* [41] who found significant increases in FBG, HbA1c and significant decreases in serum insulin and Homa-IR in wistar male rats after injecting by STZ due to cytotoxic effect of STZ. Also, STZ has a direct effect on the cell membrane permeability that resulting in failure of ionic pumps and elevated the cell size and death [41].

Supplying rats with 20%HF solution for 8 weeks resulting in pre- diabetic symptoms, which appeared as mild increases in FBG and serum insulin levels as well as Homa-IR value when compared to control values. Similar finding was recorded in Ferreira-Santos *et al* [42] and Réggami *et al* [43] who found increases in FBG, serum insulin and Homa-IR in wistar male rats after drinking HF. The effects of fructose consumption may contribute to the development of IR through different mechanisms including the loss of inhibition of gluconeogenic pathways, the increase in hepatic glucose output, the increase in insulin secretion by pancreatic islet, the down regulation of insulin receptors, adipose tissue, and muscles and the increase in proinflammatory cytokines [44].

In this study, increased intake of HFD in rat's food led to significant greater levels in serum insulin and glucose, HbA1c and Homa-IR value than in control rats (table 2). These findings are in accordance with Marques *et al* [37] Du *et al* [45] who reported that wistar and SD rats fed HFD had significant increases in FBG and insulin levels which led to develop IR although not steady hyperglycemia or diabetes. These elevations might be due to the increase in OS markers [46]. Liver disorder can happen in diabetic patients. Amplified activities of liver enzymes (ALT, AST and ALP) used as indicator of hepatocellular damage [12]. ALP has a toxicological important due to it is a marker of tissue damage by toxicants. The raise of ALP is a result of extended damage of liver cells [47]. Akhtar *et al* [12] reported that elevated ALT, AST and ALP activities are connected with IR and T2DM.

In the present study, the liver enzymes (ALT, AST and ALP) had significant elevations in alloxan, STZ and HFD groups compared to the control group. These findings are in consistent with Aslam *et al* [36], Salahshoor *et al* [14] and Marques *et al* [37] who reported different increases in the liver enzymes in alloxan, STZ and HFD induced diabetic rats respectively. The elevation of these enzymes might be due to the leakage of these enzymes from cytosol of liver cells into the bloodstream. The increase of liver enzymes may be significant markers of hepatic dysfunction. On the other hand, Aissaoui *et al* [48] suggested that the elevation in the activities of serum AST, ALT and ALP in wistar male rats may be due to the induction of hepatic dysfunction by diabetes. Wu and Yan [49] reported that after STZ is eliminated out from the body, any functional decadence of the kidney and the liver may because of the effects of hyperglycemia.

In HF drinking water group, liver enzyme activities (AST, ALT and ALP) were still within the control value which indicating the dose and duration of fructose which were used in our study fail to produce liver damage. The present results are in agreement with Bratoeva *et al* [50] who reported that there were no changes in liver enzymes after 16 weeks with 35% HF in drinking water treated rats.

In this study, in both alloxan and STZ groups, the hyperglycemia led to renal dysfunction which was indicated by significant increases in blood urea and UA as shown in (Table 4). Gupta *et al* [51] obtained similar results in albino male rats. The increase in blood urea might be due to the catabolism of nucleic acids and protein [47].

In the current study, the elevation of blood urea was detected in HF treated group. Saleh *et al* [52] recorded that the blood urea elevated in albino male rats drank HF for 8 weeks and this an indicator of renal injury.

In this study, kidney dysfunction in HFD group observed significantly by the elevations in blood urea and UA, which was similar to results in a previous study by Du *et al* [45] who recorded significant increases in urea and UA in SD male rats fed HFD. The elevation of blood urea and UA might be due to oxidative stress and mitochondrial dysfunction inducing renal injury [53] or due to IR and hyperinsulineamia [54]. Oxidative stress is caused by mitochondrial dysfunction and is strongly linked with IR and diabetes. Excessive energy supply leads to an elevation in oxidative activity and this along with the decrease in antioxidant defense that causes an overproduction of ROS in mitochondria which leads to damage in other macromolecules such as protein, lipids and nucleic acids [10]. OS happens due to the imbalance in redox between the generation of ROS and the response from the endogenous antioxidant network [55]. OS leads to a decrease in glucose transporter-4 (GLUT4) that plays a role in the regulation of the entrance of glucose into insulin dependent cells such as myocytes and adipocytes. The reduction of GLUT4 leads to reduce glucose that enters target cells and this result in insulin resistance [56]. Hyperglycemia can induce OS by sundry mechanisms including polyol pathway, glucose autoxidation, and protein advanced glycation end products (AGE) formation [57].

The present study, showed that alloxan and STZ groups induced OS which was confirmed by high significant increase in serum MDA levels, and decreases in serum activities of SOD, CAT and Gpx as well as GSH levels compared to the control group.These findings are in accordance with Sekiou *et al* [31] and Ghanbari *et al* [13] who recorded increase in MDA levels and decreases in serum activities of SOD, CAT and Gpx in wistar male rats injected by alloxan and STZ respectively. The elevation of MDA might be due to alloxan enters β-cells through GLUT2 transporters and induces glucokinase inhibition and ROS formation. ROS are generated via the redox cycle of alloxan GSH-mediated reduction into dialuric acid and then dialuric acid oxidation back to alloxan. During this process, an alloxan radical is produced either by one-electron reduction of alloxan or by one-electron oxidation of dialuric acid, together with hydrogen peroxide, the superoxide anion radical and the hydroxyl radical. Hydroxyl radicals are responsible for the necrotic death of the cells of pancreas and as a result alloxan induces low blood insulin level as well as diabetes in animal models [40].

Furthermore, HFD group induced OS which confirmed by a significant increase in serum MDA, but decreases in SOD, CAT and Gpx levels. These results are in consistent with Moreno-Fernández *et al* [10] who recorded similar results in wistar male rats. The mechanisms for elevating OS by HFD are not completely understood [58] but may be including increased mitochondrial dysfunction, fatty acid oxidation and augmented NADPH oxidase activity [59]. Antioxidant enzymes (CAT, SOD and Gpx) were reduced in alloxan and STZ groups, these results might be due to glycation of these enzymes that happened due to the increase of FBG levels [56]. According to Sekiou *et al* [31] who recorded that antioxidant enzymes decreased in wistar male rats due to the overproduced of ROS in diabetic condition induces the oxidation of proteins. Proteins oxidation would be the source of several enzymatic dysfunctions and could amplify the apoptosis of β-cells having a low enzymatic antioxidant system. This might explain the significant decreases in Gpx , SOD and CAT activities with an increase in MDA levels and a decrease in GSH in alloxan and STZ as compared to control, indicating an oxidative stress state. The reduction in SOD in diabetic wistar rats might be due to glycosylation of SOD or leakage of a cofactor required for SOD activity [60].

4. Conclusion

Both alloxan and STZ in high doses stimulated insulin insufficiency, hyperglycemia, hepatic dysfunction and OS. These results led to induce T1DM. These models are easy and suitable for studying T1DM complications and treatments. 20% High fructose in drinking water for 8 weeks led to slightly hyperglycemia, IR and hyperinsulineamia but failed to induce diabetes. These are features of pre-diabetes which need to increase dose and duration of HF to induce T2DM. 30% High fat diet for 8 weeks induced slightly hyperglycemia, IR, hyperinsulineamia, hepatic dysfunction and OS. These are features of T2DM. This model is easy and suitable for studying T2DM complications and treatments.

References

1. Aschner P, Karuranga S, James S et al. The International Diabetes Federation's guide for diabetes epidemiological studies. Diab res. Clin. Practice. 172 (2021). Doi:10.1136/ bmjdrc-2021-002122
2. DiMeglio L, Evans-Molina C and Oram R. Type 1diabetes. The Lancet. 391(2018) 2449-2462. Doi: 10.1016/S0140-6736(18)31320-5.
3. Egan A and Dinneen S. What is diabetes?. Medicine. 47(2019) 1-4. https://doi.org/10.1016/j.mpmed.2018.10.002.
4. Banday M, Sameer A and Nissar S. Pathophysiology of diabetes: An overview. Avicenna J. Medicine. 10(04) (2020) 174-188. Doi: 10.4103/ajm.ajm_53_20.
5. Kotronen A, Juurinen L, Tiikkainen M et al. Increased Liver Fat, Impaired Insulin Clearance, and Hepatic and Adipose Tissue Insulin Resistance in Type 2 Diabetes. Gastroenterology. 135(2008) 122–130. https://doi.org/10.1053/j.gastro.2008.03.021.
6. Arslanian S, Saad S, Lewy S et al. Hyperinsulinemia in african-american children:decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes. 51(2002) 3014–3019. https://doi.org/10.2337/diabetes.51.10.3014.
7. Mcintyre H, Catalano P, Zhang C et al. Gestational diabetes mellitus. Nature reviews Disease primers. 5(1) (2019) 1-19. https://doi.org/10.1038/s41572-019-0098-8.
8. Papatheodorou K, Banach M, Bekiari E et al. Complications of diabetes 2017. J. Diabetic res. (2018). https://doi.org/10.1155/2018/3086167
9. Kang Q and Chunxue Y. Oxidative stress and diabetic retinopathy: Molecular mechanisms,

pathogenetic role and therapeutic implications. Redox biology. 37(2020) 101799. https://doi.org/10.1016/j.redox.2020.101799.
10. Moreno-Fernández S, Garcés-Rimón M, Vera G et al. High fat/high glucose diet induces metabolic syndrome in an experimental rat model. Nutrients. 10(10) (2018) 1502. https://doi.org/10.3390/nu10101502
11. Islam M and Code Q. Streptozotocin is more convenient than Alloxan for the induction of Type 2 diabetes. IJPR, 7(1) (2017).https://dx.doi.org/10.7439/ijpr.v7i1.3790
12. Akhtar N, Akram M, Daniyal M and Ahmad S. Evaluation of antidiabetic activity of Ipomoea batatas L. extract in alloxan-induced diabetic rats. J. Immuno. Pharma. 32(2018). DOI: 10.1177/2058738418814678
13. Ghanbari E, Nejati V and Khazaei M. Improvement in serum biochemical alterations and oxidative stress of liver and pancreas following use of royal jelly in streptozotocin-induced diabetic rats. Cell J. Yakhteh. 18(3) (2016) 362. Doi: 10.22074/cellj.2016.4564
14. Salahshoor M, Mohammadi M, Roshankhah S et al. Effect of *Falcaria vulgaris* on oxidative damage of liver in diabetic rats. J. Diab. Metab. 18 (2019) 15-23. https://doi.org/10.1007/s40200-019-00385-3
15. Elseweidy M, Amin R, Atteia H and Aly M. Nigella sativa oil and chromium picolinate ameliorate fructose-induced hyperinsulinemia by enhancing insulin signaling and suppressing insulin-degrading enzyme in male rats. Bio. Trace Element Res. 184(1) (2018) 119-126. DOI 10.1007/s12011-017-1167-z.
16. Gheibi S, Kashfi K and Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed. Pharma. 95(2017) 605-613. DOI: 10.1016/j.biopha.2017.08.098.
17. Barham D and Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 97(1151) (1972) 142-145. DOI: 10.1039/AN9729700142
18. Temple R, Clark P and Hales C. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Dia. Med. 9(6) (1992) 503-512. https://doi.org/10.1111/j.1464-5491.1992.tb01830.x
19. Hoelzel W, Weykamp C, Jeppsson J *et al.* IFCC Working Group on HbA1c Standardization, IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin. Chem. 50(1) (2004) 166-174. https://doi.org/10.1373/clinchem.2003.024802
20. Huang X, Choi, Y, Im H, Yarimaga O et al. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors. *6*(7) (2006) 756-782. https://doi.org/10.3390/s6070756
21. Tietz N, Burtis C, Duncan P et al. A reference method for measurement of alkaline phosphatase activity in human serum. Clin. Chem. *29*(5) (1983) 751-761. https://doi.org/10.1093/clinchem/29.5.751
22. Orsonneau J, Massoubre C, Cabanes M, Lustenberger P. Simple and sensitive determination of urea in serum and urine. Clin. Chem. (1997) 38(5): 619-623. https://doi.org/10.1093/clinchem/38.5.619
23. Mohabbati-Kalejahi E, Azimirad V, Bahrami M, Ganbari A. A review on creatinine measurement techniques. Talanta. (2012) 97: 1-8. https://doi.org/10.1016/j.talanta.2012.04.005
24. Zhao Y, Yang X, Lu W, Liao H et al. Uricase based methods for determination of uric acid in serum. Microchimica Acta. (2009) 164: 1-6. DOI 10.1007/s00604-008-0044-z
25. Wallin B, Rosengren B, Shertzer H and Camejo G. Lipoprotein oxidation and measurement of TBARS formation in single microlitre plate; it's use for evaluation of antioxidants. Anal. Biochem. 208 (1) (1993) 10-15. https://doi.org/10.1006/abio.1993.1002
26. Exner C, Hebebrand J, Remschmidt H et al. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. Molecular psychiatry, 5(5) (2000) 476-481. https://doi.org/10.1038/sj.mp.4000771
27. Arthur J and Boyne R. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficien cattle. Life sciences. 36(16) (1985) 1569-1575. https://doi.org/10.1016/0024-3205(85)90381-9
28. Sinha A. Colorimetric assay of catalase. Analytical biochemistry. 47(2) (1972) 389-394. https://doi.org/10.1016/0003-2697(72)90132-7
29. Paglia D and Valentine W, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. lab. Clin. Med. 70(1) (1967) 158-169. https://doi.org/10.5555/uri:pii:0022214367900765
30. Matthews D, Hosker J, Rudenski A et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28 (1985) 412–419. https://doi.org/10.1007/BF00280883
31. Sekiou O, Boumendjel M, Taibi F et al. Mitigating effects of antioxidant properties of Artemisia herba alba aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. Archives of physiology and biochemistry. 125(2) (2019) 163-173. https://doi.org/10.1080/13813455.2018.1443470
32. Bagheri S, Khorramabadi R, Assadollahi Vet al. The effects of pomegranate peel extract on the gene expressions of antioxidant enzymes in a rat model of alloxan-induced diabetes. Arch. Phys. Bio, 129(4) (2023) 870-878. https://doi.org/10.1080/13813455.2021.1877308

rat. Indian J. Pharma. Educa. Res. 51(4) (2017) 661-6. DOI: 10.5530/ijper.51.4s.96

34. Ul Haq M, Shah G, Gul A et al. Biogenic Synthesis of Silver Nanoparticles Using Phagnalon niveum and Its In Vivo Anti-Diabetic Effect against Alloxan-Induced Diabetic Wistar Rats. Nano. 12(5) (2022) 830. Doi.org/10.3390/nano12050830
35. Samadi-Noshahr Z, Hadjzadeh M, Moradi-Marjaneh R and Khajavi-Rad A. The hepatoprotective effects of fennel seeds extract and trans-Anethole in streptozotocin-induced liver injury in rats. Food sci. nutrition. 9(2) (2021) 1121-1131. https://doi.org/10.1002/fsn3.2090
36. Aslam B, Hussain A, Sindhu Z et al. Polyphenols-rich polyherbal mixture attenuates hepatorenal impairment, dyslipidaemia, oxidative stress and inflammation in alloxan-induced diabetic rats. J. Applied Animal Res. 51(1) (2023) 515-523. https://doi.org/10.1080/09712119.2023.2230754
37. Marques C, Meireles M, Norberto S et al. High-fat diet-induced obesity Rat model: a comparison between Wistar and Sprague-Dawley Rat. Adipocyte. 5(1) (2016) 11-21. https://doi.org/10.1080/21623945.2015.106172
38. American Diabetes Association (ADA). Standards of medical care in diabetes 2010. Diabetes care. 33 (2010). Doi: 10.2337/dc10-S011
39. Antunes L, Elkfury J, Jornada M et al. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch. Endocrinol. Metab. 60 (2016) 138-142. https://doi.org/10.1590/2359-3997000000169
40. Mistry J, Biswas M, Sarkar S and Ghosh S. Antidiabetic activity of mango peel extract and mangiferin in alloxan-induced diabetic rats. Future J. Pharm. Sci. 9(1) (2023) 1-13. Doi.org/10.1186/s43094-023-00472-6
41. Peña-Montes D, Huerta-Cervantes M, Ríos-Silva M et al. Effects of dietary iron restriction on kidney mitochondria function and oxidative stress in streptozotocin-diabetic rats. Mitochondrion. 54 (2020) 41-48. https://doi.org/10.1016/j.mito.2020.07.001
42. Ren L. Protective effect of ganoderic acid against the streptozotocin induced diabetes, inflammation, hyperlipidemia and microbiota imbalance in diabetic rats. Saudi J. Bio. Sci. 26(8) (2019) 1961-1972.https://doi.org/10.1016/j.sjbs.2019.07.005
43. Ferreira-Santos P, Aparicio R, Carrón R et al. Lycopene-supplemented diet ameliorates metabolic syndrome induced by fructose in rats. J. Fun. Foods. 73 (2020)104098. https://doi.org/10.1016/j.jff.2020.104098.
44. Réggami Y, Benkhaled A, Boudjelal A et al. Artemisia herba-alba aqueous extract improves insulin sensitivity and hepatic steatosis in rodent model of fructose-induced metabolic syndrome. Arch. Physio. Biochem. 127(6) (2021) 541-550. https://doi.org/10.1080/13813455.2019.1659825
45. Farag M, Ashour E and El-Hadidy W, Amelioration of high fructose diet-induced insulin resistance, hyperuricemia, and liver oxidative stress by combined use of selective agonists of PPAR-α and PPAR-γ in rats. Dubai Med J. 3(2020) 76–86. https://doi.org/10.1159/000506899
46. Du H, Wang Q and Yang X. Fu brick tea alleviates chronic kidney disease of rats with high fat diet consumption through attenuating insulin resistance in skeletal muscle. J. Agricultural. Food. chem. 67(10) (2019) 2839-2847. https://doi.org/10.1021/acs.jafc.8b06927
47. Maciejczyk M, Żebrowska E, Zalewska A and Chabowski A. Redox balance, antioxidant defense, and oxidative damage in the hypothalamus and cerebral cortex of rats with high fat diet-induced insulin resistance. Oxi. Med. Cellular Longevity. (2018). https://doi.org/10.1155/2018/6940515
48. Preetha P, Devi V and Rajamohan T. Comparative effects of mature coconut water (*Cocos nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. Revista Brasileira de Farmacognosia. 23(3) (2013) 481-487. https://doi.org/10.1590/S0102-695X2013005000027
49. Aissaoui O, Amiali M, Bouzid N et al. Effect of Spirulina platensis ingestion on the abnormal biochemical and oxidative stress parameters in the pancreas and liver of alloxan-induced diabetic rats. Pharma. Bio. 55(1) (2017) 1304-1312. https://doi.org/10.1080/13880209.2017.1300820
50. Wu J and Yan L. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Dia. MetS. Obesity: targets and therapy. (2015) 181-188. https://doi.org/10.1007/978-3-030-31403-3_9.
51. Bratoeva K, Nikolova S, Merdzhanova A et al. Association between serum CK-18 levels and the degree of liver damage in fructose-induced metabolic syndrome. Metabolic syndrome and related disorders. 16(7) (2018) 350-357. https://doi.org/10.1089/met.2017.0162
52. Gupta A, Kumar R and PandeyA. Antioxidant and antidiabetic activities of *Terminalia bellirica* fruit in alloxan induced diabetic rats. South African J. Botany. 130(2020) 308-315. https://doi.org/10.1016/j.sajb.2019.12.010
53. Saleh R, Merghani B, Awadin W. Effect of high fructose administration on histopathology of kidney, heart and aorta of rats. J. Advanced Veterinary and Animal Research. 4(1) (2017) 71-79. http://doi.org/10.5455/javar.2017.d193
54. Sun Y, Ge Y, Li X et al. High-fat diet promotes renal injury by inducing oxidative stress and mitochondrial dysfunction. Cell death & disease. 11(10) (2020) 914. https://doi.org/10.1038/s41419-020-03122-4.
55. Roza N, Possignolo L, Palanch A and Gontijo A. Effect of long-term high-fat diet intake on peripheral insulin sensibility, blood pressure, and renal function in female rats. Food. Nutr. Res. 60(1) (2016) 28536. https://doi.org/10.3402/fnr.v60.28536
56. Al-Attar A, Alsalmi F. Effect of *Olea europaea* leaves extract on streptozotocin induced diabetes in male albino rats. Saudi J. Bio Sci. 26(1) (2019) 118-128.https://doi.org/10.1016/j.sjbs.2017.03.002

57. Hurrle S and Hsu W. The etiology of oxidative stress in insulin resistance. Biomed. J. 40(5) (2017) 257-262. https://doi.org/10.1016/j.bj.2017.06.007
58. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. J. Dia. 6(3) (2015) 456. Doi: 10.4239/wjd.v6.i3.456
59. Kesh S, Sarkar D and Manna K. High-fat diet-induced oxidative stress and its impact on metabolic syndrome: a review. Asian J. Pharm. Clin. Res. 9(1) (2016) 47-52.
60. Le Lay S, Simard G, Martinez M and Andriantsitohaina R. Oxidative stress and metabolic pathologies: from an adipocentric point of view. Oxid. Med. Cellular longevity. (2014). https://doi.org/10.1155/2014/908539
61. Ayepola O, Brooks N, and Oguntibeju O. Kolaviron improved resistance to oxidative stress and inflammation in the blood (erythrocyte, serum, and plasma) of streptozotocin-induced diabetic rats. The Scientific World. J. (2014). https://doi.org/10.1155/2014/921080