BACKGROUND: Diabetes mellitus, as a chronic metabolic disease, is characterized mainly by hyperglycemia due to insulin deficiency. The cornerstone of diabetes research is the experimental induction of diabetes in laboratory animals, thus various approaches were applied for induction. Objective: The aim of this study was the evaluation of different methods applied to the induction of diabetes in experimental animals. Methods: Alloxan (200 mg/kg of body weight), streptozotocin (50mg/kg body weight) and a high carbohydrate diet were applied in rats, for one month. 45 male albino rats at age 2-4 months and weight (220-260g) were gathered into 5 groups (9 rats for each group). Body physiological parameters and serum glucose, insulin, insulin resistance, and serum lipid profile were estimated. Results: Outcomes reveal that the body weight and body mass index were increased in treated groups, as well as, the greatest serum glucose, minimum serum insulin, and insulin resistance were in the group of the streptozotocin+diet. Whereas the maximum lipid profile fluctuation was in the group of the Alloxan+diet. Conclusion: It could be concluded that the different approaches of experimentally induction of diabetes in rats have a diverse degree of impact on the physiological and biochemical profiles.

Keywords: Alloxan, Diabetes mellitus, Diet, Rats, Streptozotocin.
and neuropathy, as well as, in the fluctuations in the body weight, and body mass index [9, 10]. Configurations of dyslipidemia in DM and involving principal threat factors have been defined in many reports [11-13]. Atherosclerosis-provoked heart disease, stroke, and hypertension are the chief causes of the growing rate of mortalities among DM patients [14,15]. Although relative or absolute insulin deficiency is prevailing, there is a wide range of variations in etiology and pathophysiology of DM. Thus a long journey of DM research began many decades ago and is still continuous [16]. On the other hand, the milestone of DM research is the experimental induction of DM in lab animals; by streptozotocin, alloxan, and other specific substance, and other methods (such as pancreatectomy) [17]. So we conduct this project to evaluate a common induction method of DM by using, stereotopsotizen, alloxan and specific diet approaches in rats.

Experimental

Experimental Animals

Forty-five male albino rats at age 2-4 months and weight (220-260g) were used in this project, all experimental animals appear healthy and manipulated under suitable and ethical properties in a laboratory animals house, College of Veterinary Medicine, University of Mosul. Rats were set in special breeding cages for rat (dimensions of 17X20X50cm) and environmental conditions were identical for rats, the temperature of 22±2 °C, dark/light cycle 12/12 hours, stander diet (except for candidate diet group) for rats and water ad libitum [18,19]. Rats were divided into the following groups:

1- Control group:
Nine rats were considered a control group and not treated with any chemicals or foods.

2- Alloxan group:
Nine rats were injected subcutaneously (one time) with alloxan (Ayonchen, UK) 200 mg/kg of body weight [20]. The level of glucose was measured frequently by using Accu-Chek® device (Roche, Germany).

3- Streptozotocin (STZ) group:
Nine rats were injected subcutaneously with STZ (Direvo industrial biotechnology, Germany) 50mg/kg body weight [20], weekly for one month.

4- Alloxan+diet group:
Nine rats were injected subcutaneously (one time) with alloxan 200 mg/kg and given white bread only as a food with saturated fructose solution 40% W/V as a drinking water for one month.

5- STZ+diet group:
Nine rats were injected subcutaneously with STZ 50 mg/kg body weight weekly and given white bread with saturated fructose solution 40% W/V as a drinking water, for one month.

Body physiological parameters:
An electronic weighing scale with a 0.01 g accuracy has been utilized, whereas abdominal circumference, and body length have been determined by scale tape at the start and end of each experiment. From the nose to the origin of the tail was considered the body length [21]. Abdominal circumference was measured around the anterior region of the abdomen. Anyway, the body mass index (BMI) was calculated as BMI=body weight(g)/ body length² (cm²) [22].

Biochemical tests:
In all groups, blood was collected by a heparinized capillary tube from the retro-ocular vein of the eye [23], and then serum was separated by centrifugation (3000xg) for 15 min. The serum was kept at -20 °C for biochemical analysis. Serum insulin level was determined by using a sandwich-ELISA kit (Elabscience, USA) specific for rats, and a microtiter reader (Biotek, USA) was used at 450 nm. Serum glucose level was determined by enzymatic (oxidase-peroxidase) method, by using a kit (Biolab, France) and the concentration was determined at 505 nm by spectrophotometer (Vispectorhophometor, China). From the data of insulin and glucose levels (as mentioned above), insulin resistance was estimated mathematically as follows [24]:

$$HOMA-IR^* = \frac{\text{Serum glucose} \times \text{Serum insulin}}{22.5}$$

*HOMA-IR (Homeostatic Model Assessment for Insulin Resistance)

Serum lipid profile:
Serum total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein (HDL), low-density lipoprotein–cholesterol (LDL-C), and
serum very low-density lipoprotein-cholesterol (VLDL-C) were measured by using an enzymatic colorimetric method, (Biolabe, France) and the concentration determined at 500 nm by using the spectrophotometer (Vispectrophotometer, China) (24). While atherogenic index (AI) was determined as follows [25]:

\[ \text{AI} = \frac{\text{TC} - \text{HDL}}{\text{HDL}} \]

**Statistical analysis**

All experimental data were analyzed statistically by applying SPSS (ver.25) to determine mean ± S.D, and for measuring significance ANOVA tests were used and the results were considered significant when \( p \leq 0.05 \) [26].

**Results and Discussion**

Successfully, diabetes mellitus was induced in albino rats by alloxan, STZ, and a special diet. Induction of diabetes conducted by the direct impact of the beta cell of the islet of Langerhans [13]. The precise mechanism of diabetogenic action of alloxan consider mainly on selective attack of beta cell by oxidation of –SH groups, glucokinase inhibition, intracellular calcium imbalance, and free radical formation [14]. The selectivity of alloxan to the beta cells may be due to a high carbohydrate in food and drinking water which contributed to the high body weight gain [27]. Also remarkably, the rats which have injected with alloxan and STZ have a low body weight when compared with the control group (Table 1). This result may be due to that diabetes causes a deterioration in most body systems leading to body weight loss because of the inability of most body cells to utilize glucose (although it is at a higher level in the serum) with default insulin [27,28]. Accordingly, the body mass index was greater significantly (\( p \leq 0.05 \)) in STZ+diet and alloxan+diet groups, and lesser in alloxan and STZ groups, when compared with the control group.

On the other hand, the greatest serum glucose level was in the group of STZ+diet (244.79 mg/dl ±9.7) (Table 2), and there was a significant difference (\( p \leq 0.05 \)) between all treated groups when compared with the control group. In the same table 2, the greatest level of serum Insulin was in the same group (STZ+diet). In the same manner, HOMA-IR and HOMA-B index was lowest in group STZ+diet. So our results agree with many authors [16] who induced diabetes in rats by injection of alloxan. Also, our findings may be due to the synergistic effect of both approaches in which diabetes status (induced by alloxan) was exacerbated by a diabetogenic diet [17, 29].

As well as, the level of serum insulin (Table 2) confirms the above hypothesis.

However, lipid profiles (Table 3) reveal a greater elevation of cholesterol in the group alloxan+diet (240.75 ± 15.89), also all treated groups have a significantly (\( p \leq 0.05 \)) higher

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**TABLE 1. Body physiological parameters.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Length (cm)</th>
<th>BMI</th>
<th>Abdominal circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>276±19c</td>
<td>22.2±1.1a</td>
<td>0.56±0.12b</td>
<td>18.6±1.40b</td>
</tr>
<tr>
<td>Alloxan</td>
<td>251±17d</td>
<td>22.6±1.3a</td>
<td>0.49±0.14a</td>
<td>17.8±1.40b</td>
</tr>
<tr>
<td>STZ</td>
<td>248±17d</td>
<td>22.4±1.0a</td>
<td>0.49±0.12c</td>
<td>17.0±1.40b</td>
</tr>
<tr>
<td>Alloxan+diet</td>
<td>362±20b</td>
<td>22.9±1.1a</td>
<td>0.69±0.10a</td>
<td>21.7±1.20a</td>
</tr>
<tr>
<td>STZ+diet</td>
<td>422±21a</td>
<td>23.5±1.6a</td>
<td>0.76±0.15a</td>
<td>22.9±1.30a</td>
</tr>
</tbody>
</table>

-All rats in this group have an initial weight of 220±10 g.
-Each value represents mean±S.D
-Different letters within each column means a significant difference (\( p \leq 0.05 \)) between groups.
cholesterol level when compared with the control
group. In the same manner, serum triacylglycerol
and LDL are greater in a group of alloxan+diet.
(Table 3). Consequently, serum HDL level was the
lowest in the group of alloxan among all groups,
while the greatest serum VLDL was in the group
of STZ (59.48 ± 5.3). All treated groups have a
significant difference (p ≤0.05) when compared
with the control group. The strong association
between diabetes and dyslipidemia is confirmed by
many authors [30-34]. Thus, hypertriglyceridemia
may be considered the principal irregularity of fat
metabolism in diabetes, and this may be due to a rise
of the triglyceride-carrying lipoproteins, VLDL,
and chylomicrons [35]. The crucial pathogenesis
for hypertriglyceridemia is the diminished
derivatization of VLDL and the chylomicron-
clearance, which is caused by reduced activity of
the enzyme lipoprotein lipase. Therefore, serum
total cholesterol level is significantly raised when
there is poor metabolic control, LDL level can
be increased and HDL decreased in reliance on
the metabolic control [36,37]. Other authors
suggested that hypertriglyceridemia is amplified
VLDL and triacylglycerol formation in the liver
particularly due to improved free fatty acids (FFA)
flux. Furthermore, the activity of the enzyme
lipoprotein lipase can be declined [38].

**Conclusion**

From this study, it could be concluded that the
different approaches for experimental induction
of diabetes in rats have a diverse degree of impact,
according to the method, on the physiological
parameters, biochemical and lipid profiles. It seems
that STZ+diet method of the induction of diabetes be
more elevating the body weight and serum glucose,
while the induction of diabetes by alloxan+diet could
be more compelling in causing dyslipidemia. As
well as, depending on the results of the lipid profile,
especially in the group of alloxan+diet, this model
can be applied in the research of cholesterol-lowering
drugs in diabetes.

**Acknowledgement**

The authors would like to thank the College
of Veterinary Medicine, University of Mosul for
their non-financial support of this project.

**Conflicts of Interest**

The authors declare that they have no
competing interests.

**Funding statement**

The authors declare that this study has not
received any financial support.

**TABLE 2. Biochemical Parameters of control and treated groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum glucose mg/dl</th>
<th>Insulin mg/dl</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.66±6.2</td>
<td>20.22±2.0</td>
<td>67.99±0.55</td>
</tr>
<tr>
<td>Alloxan</td>
<td>166.87±12.4</td>
<td>10.1±1.1</td>
<td>74.90±0.606</td>
</tr>
<tr>
<td>STZ</td>
<td>184.32±5.5</td>
<td>12.4±1.8</td>
<td>101.58±0.44</td>
</tr>
<tr>
<td>Alloxan+diet</td>
<td>204.80±11.2</td>
<td>8.75±1.0</td>
<td>79.58±5.4</td>
</tr>
<tr>
<td>STZ+diet</td>
<td>244.79±9.7</td>
<td>33.92±2.7</td>
<td>36.9±1.8</td>
</tr>
</tbody>
</table>

-Each value represents mean±S.D
-Different letters within each column means a significant difference (p≤0.05) between groups.

**TABLE 3. Lipid profile of control and treated groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/ dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/ dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.48±9.7</td>
<td>66.90±3.9</td>
<td>45.53±1.9</td>
<td>34.45±6.1</td>
<td>18.20±1.6</td>
<td>0.57±0.1</td>
</tr>
<tr>
<td>Alloxan</td>
<td>144.82±12.3</td>
<td>120.23±4.4</td>
<td>17.77±4.8</td>
<td>96.90±5.7</td>
<td>48.38±3.4</td>
<td>7.14±1.1</td>
</tr>
<tr>
<td>STZ</td>
<td>180.50±14.7</td>
<td>92.88±4.5</td>
<td>23.24±1.3</td>
<td>91.88±3.5</td>
<td>59.48±5.3</td>
<td>6.76±1.2</td>
</tr>
<tr>
<td>Alloxan+diet</td>
<td>240.75±18.8</td>
<td>117.60±6.2</td>
<td>30.50±1.7</td>
<td>135.95±11.9</td>
<td>37.44±4.2</td>
<td>6.89±1.1</td>
</tr>
<tr>
<td>STZ+diet</td>
<td>172.40±13.6</td>
<td>107.40±4.1</td>
<td>29.44±1.8</td>
<td>123.70±10.7</td>
<td>29.34±2.4</td>
<td>4.85±0.9</td>
</tr>
</tbody>
</table>

-Each value represents mean±S.D
-Different letters within each column means a significant difference (p≤0.05) between groups.

References


يتميز داء السكري، باعتباره مرض ايضي مزمن، بارتفاع كلوكوز الدم بسبب نقص الأنسولين. يعد استحداث داء السكري أهم جزء عند اجراء البحوث التجريبية لداء السكري، لذلك استخدمت طرق مختلفة لاستحداث داء السكري في الحيوانات المختبرية. كان الهدف من الدراسة الحالية هو تقييم الطرق المختلفة لاستحداث داء السكري في جوانات التجربة. تم استخدام كل من الالوكسان والستربتوزوتسون غذاء عالي الكربوهيدرات في ذكور الجرذان البالغة. وأظهرت نتائج الدراسة وجود زيادة في وزن الجسم ومعامل كتلة الجسم، كما أن أعلى مستوى من الكلوكوز في الدم، وأقل مستوى من الأنسولين، ومقاومة الأنسولين كانت في مجموعة الستربتوزوتسون+ الغذاء الخاص. بينما التذبذب في مستوى شحوم الدم كان في مجموعة الالوكسان + الغذاء الخاص. ويمكن الاستنتاج من هذه الدراسة أن الطرق المختلفة لالتهاب داء السكري تجريبياً في الجرذان لها درجات متنوعة من التأثير على القيم الكيميائية الحيوية.

الكلمات الدالة: داء السكري، الالوكسان، الستربتوزوتسون، الغذاء، الجرذان.

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