

## The Effects on Offspring of Diabetes Mellitus Induced by Chemical Agents in Rats with Advanced Pregnancy

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### ABSTRACT

**Background:** Gestational diabetes mellitus is an increasingly frequent metabolic disorder that is important for both baby and mother. New studies on the development and treatment of the disease are required.

**Objective:** To investigate the effects on offspring's survival and the biochemical values of diabetes mellitus, induced by different doses of two chemical agents among 35 rats with advanced pregnancy.

**Methods:** The rats were randomly divided into five groups, with the rats in Group 1 as the control group. Alloxan was administered intraperitoneally at doses of 40 and 60 mg/kg in Groups 2 and 3, respectively. Streptozotocin was injected intraperitoneally at doses of 40 and 60 mg/kg in Groups 4 and 5, respectively. Deliveries were monitored, and offspring numbers, survival rates and congenital anomalies were recorded. At the end of the study, blood was drawn from one female offspring in each group; glucose, total protein, albumin, triglyceride, cholesterol, calcium and phosphorus levels were measured, and inter-group comparisons were made. Diabetic agents administered at various doses prolonged the duration of pregnancy.

**Results:** Offspring's deaths were most frequent in the alloxan groups. The number of offspring mortalities in the streptozotocin group was higher than that of the control group, but lower than that of the alloxan group. No differences in glucose, total protein, albumin, triglyceride, cholesterol, calcium and phosphorus levels were observed between the groups. These results indicate that the female offspring, born from rats with gestational diabetes mellitus induced by different chemicals, were only clinically affected. No effect of the type of chemicals on the results was found.

**Conclusion:** The use of streptozotocin in the studies on female offspring born from rats with gestational diabetes mellitus is recommended.

**Keywords:** Alloxan, diabetes mellitus, offspring, rat, streptozotocin

### INTRODUCTION

Hyperglycaemia and hyperketonaemia have been observed to cause foetal malformations in experiments conducted with rats. In these tests, the malformation rate was 20% when the glucose level was twofold higher than the control level and 50% when the glucose level was fourfold higher than the control level (1–3).

Diabetes may be induced using surgical, pharmacological or genetic methods in various types of

animals. Rats are currently the most commonly used test animal model (4–6). A pharmacological induction model has been used to induce diabetes in most studies. Streptozotocin and alloxan are the most commonly used chemicals. These substances exert their diabetic effect after injection *via* the intraperitoneal, intravenous and subcutaneous routes. The dose of the agents varies depending on the type of animal and the administration route of the agents (7, 8). In studies of diabetes, rats with

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fasting plasma glucose levels above 250 mg/dL are considered diabetic (9).

Hypoinsulinaemia syndrome induced by streptozotocin is defined as streptozotocin diabetes. Diabetes is expected to develop on the same day of administration of streptozotocin (10). Alloxan is a selective agent that damages pancreatic beta cells. Pancreatic beta cells specifically take up alloxan, leading to the accumulation of this agent inside the cell (7).

The present study aimed to determine the influence of gestational diabetes induced using different chemical agents on newborns.

## MATERIALS AND METHODS

### Animals

In this study, 35 female Wistar albino rats weighing 200–250 g and aged 3–4 months, were used as the animal model. The rats were purchased and kept in the animal facility of the Firat University Experimental Research Unit, Elazig, Turkey, in groups, in cages with 12 h:12 h light-dark regimen and food and water *ad libitum*. Ethics committee approval was obtained from the Firat University Test Animals Ethics Committee (FÜHADEK 2013/07-96).

### Vaginal irrigation

Vaginal irrigation was applied as described by Risvanli *et al* (11).

### Application groups

On day 13 of pregnancy, the animals were grouped as follows: Group 1 was the control group which received the intraperitoneal saline solution ( $n = 7$ ). Group 2 received 40 mg/kg of alloxan (Sigma) intraperitoneally ( $n = 7$ ) (12). Group 3 received 60 mg/kg of alloxan (Sigma) intraperitoneally ( $n = 7$ ) (12). Group 4 received 40 mg/kg of streptozotocin (Sigma) intraperitoneally ( $n = 7$ ) (13). Group 5 received 60 mg/kg of streptozotocin (Sigma) intraperitoneally ( $n = 7$ ) (13).

Blood was drawn from the tails of the animals on day 3 of injections, which corresponded to day 16 of gestation, and glucose concentrations were measured using a glucometer. While a normal plasma glucose value was accepted as 90–110 mg/dL, animals with plasma glucose levels above 250 mg/dL were accepted as diabetic (9).

### Analyses

When the offspring were one month of age, one healthy female offspring of each animal was decapitated. Blood was drawn from the decapitated animals, and sera were

separated following routine procedures and stored at  $-20^{\circ}\text{C}$  until the day of measurements.

Glucose, total protein, albumin, triglyceride, cholesterol, Ca and P levels were measured, and inter-group comparisons were made. The measurements were made using the spectrophotometric method.

### Statistical analysis

The Kruskal–Wallis test was utilized to compare the duration of pregnancy and serum glucose, total protein, albumin, triglyceride, cholesterol, Ca and P levels between the groups. The Mann–Whitney  $U$  test was used to determine the significance level. The Chi-square test was applied to determine the survival rates. The statistical analyses were performed using the SPSS 11.5 software program.

## RESULTS

The mean plasma glucose concentrations of the mother rats on day 16 of pregnancy are presented in Table 1. One animal in each of Groups 2, 3 and 5 died after the injections. One animal in each of Groups 2 and 3 had abortions (Table 2).

Table 1: Blood glucose concentrations of rats on the 16th day of pregnancy

Group	Glucose (mg/dL)
Group 1 ( $n = 7$ )	118.00 $\pm$ 12.01
Group 2 ( $n = 7$ )	263.71 $\pm$ 9.30
Group 3 ( $n = 7$ )	283.29 $\pm$ 28.14
Group 4 ( $n = 7$ )	344.86 $\pm$ 85.12
Group 5 ( $n = 7$ )	356.86 $\pm$ 70.14

Blindness was observed in one offspring born to the rats in Group 4. No anomalies were observed in the other groups (Table 2).

Table 2: Distribution of the results with pregnancy and offspring survival among the groups.

Groups	Gestation time (days)	Offspring survival rate			NOA	Abort
		NO	NSO	NOD		
Group 1 ( $n = 7$ )	21.00 $\pm$ 0.31a	68	67a	1a	0	0
Group 2 ( $n = 6$ )	22.20 $\pm$ 0.37b	43	13b	30b	0	1
Group 3 ( $n = 6$ )	22.00 $\pm$ 0.0b	41	14c	27c	0	1
Group 4 ( $n = 7$ )	22.43 $\pm$ 0.20b	62	55d	7d	1	0
Group 5 ( $n = 6$ )	22.83 $\pm$ 0.07b	45	41ad	4ad	0	0
p	*	**	**			

NO = Number of offspring, NSO = Number of surviving offspring, NOD = Number of deceased offspring, NOA = Number of offspring anomalies \* $p < 0.05$ ; \*\* $p < 0.01$

Different letters in the same column indicate significant differences between the values.

The inter-group comparisons revealed that the duration of pregnancy was shorter in the control group (*ie*, Group 1) than in the experimental groups ( $21.00 \pm 0.31$  days) and that diabetic agents administered at different doses prolonged the duration of pregnancy ( $p < 0.05$ ) (Table 2).

When the groups were compared with respect to survival rates, death was most commonly observed in the alloxan groups (30 in Group 2 and 27 Group 3) ( $p < 0.01$ ); see Table 2. In the streptozotocin-treated groups, the number of offspring mortalities was higher (7 in Group 4 and 4 in Group 5) than that of the control group, but lower than that of the alloxan groups ( $p < 0.01$ ); see Table 2.

The biochemical parameters of the offspring are summarized in Table 3. However, no differences in serum glucose, total protein, albumin, triglyceride, cholesterol, Ca and P values were observed between the groups ( $p > 0.05$ ).

## DISCUSSION

Some foetal and maternal complications were observed more frequently in pregnancies complicated with diabetes, and many pregnancies were terminated in the early weeks due to these complications. In addition, unexplained foetal losses are observed at advanced weeks of gestation (14–16). In the present study, different types of chemical agents were determined to cause changes in the survival of offspring. A total of 57 rats in the alloxan-treated groups and 11 rats in the streptozotocin-treated groups died. One offspring died in the control group. However, streptozotocin caused a smaller number of offspring mortality than alloxan. Furthermore, in this study, a prolonged duration of pregnancy was observed, and stillbirth, abortion, blindness, and maternal death were observed more frequently in diabetic rats than in non-diabetic rats. The duration of pregnancy was shorter in the control group, that is, in Group 1 ( $21.00 \pm 0.31$  days) than in the other groups, and diabetes inducing agents

applied at various doses prolonged the duration of pregnancy ( $p < 0.05$ ). High abortion rates were encountered in the treatment groups.

Congenital anomalies, which are observed at a rate of 1–2% in humans, are four- to eightfold more frequent among patients who have known pregestational diabetes; this condition is an important cause of mortality in diabetic pregnancies (17–19). In animal studies, Lopez-Soldado and Herrera (20) reported that there are concerns that streptozotocin-induced Type 1 diabetic pregnancy also leads to macrosomia in offspring. The streptozotocin, when administered at a high single dose at the advanced pregnancy stage induced a lot of genetic problems. However, low doses are less common for such problems (21). In the present study, blindness was observed in only one offspring, which had received 40 mg of streptozotocin.

The rates of hypoglycaemia, hypocalcaemia, hyperbilirubinemia and polycythaemia have been reported to increase during the newborn period in babies born to diabetic mothers (22, 23). Van Assche *et al* (24) reported that animals with perinatal hyperinsulinemia display an impaired glucose tolerance at adulthood only, under high glucose. In animal models, Type 1 diabetic pregnancy in rats is associated with a significantly increased triglyceride. In the present study, no differences in the serum glucose, total protein, albumin, triglyceride, cholesterol, Ca and P levels of female offspring born to diabetic rats with advanced pregnancy were detected.

In some previous studies, streptozotocin could induce chronic diabetes in experimental animals. However, the same studies also noted that streptozotocin soon induced diabetes (20, 21, 24). In the present study, streptozotocin had established diabetes in a short time and only developing gestational diabetes in animals was included in the study.

In conclusion, these results indicate that the offspring of diabetic rats with advanced pregnancy were clinically

Table 3: Biochemical values of offspring

Groups	Glucose, (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Ca (mg/dL)	P (mg/dL)
Group 1 (n = 7)	141.86 ± 5.14	4.89 ± 0.10	3.26 ± 0.04	93.71 ± 19.42	70.14 ± 4.67	10.17 ± 1.69	6.99 ± 0.1
Group 2 (n = 5)	106.40 ± 14.98	4.87 ± 0.16	3.24 ± 0.07	56.40 ± 5.33	62.60 ± 3.61	10.83 ± 0.28	8.63 ± 0.83
Group 3 (n = 5)	129.80 ± 9.19	4.17 ± 0.29	3.20 ± 0.30	56.60 ± 13.10	51.00 ± 6.23	10.71 ± 0.32	7.56 ± 0.55
Group 4 (n = 7)	129.00 ± 5.85	4.74 ± 0.07	3.24 ± 0.04	78.00 ± 13.99	56.57 ± 2.99	11.39 ± 0.15	7.36 ± 0.53
Group 5 (n = 6)	138.00 ± 1.03	4.88 ± 0.07	3.26 ± 0.04	78.50 ± 8.72	58.50 ± 1.63	11.75 ± 0.20	6.96 ± 0.44
<i>p</i>	–	–	–	–	–	–	–

– Difference between groups is not significant ( $p > 0.05$ ).

affected. Various degrees of glucose intolerance were demonstrated to cause problems in pregnancy, as well as increased foetal, maternal and neonatal complications. In this study, conducted using rats, no striking differences were encountered in the laboratory findings, except for a prolonged duration of pregnancy, anomalous deliveries, stillbirths and abortions in the treatment groups.

Based on these data, it is again suggested that streptozotocin could be used more effectively than alloxan in the studies on female offspring born from rats with gestational *diabetes mellitus*. Also, it was found to not affect the results of the streptozotocin dose.

## ACKNOWLEDGEMENTS

This paper was summarized from the first author's master thesis.

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