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EFFECTS OF COLOSTRUM, VIRGIN AND MULTIPARA CAMEL MILK IN SPERM COUNT AND SPERM DEFORMITY OF DIABETIC RATS

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Abstract

The present study was conducted to investigate the effect of colostrums and camel milk treatment on sperm count and sperm deformity of alloxan induced diabetic rats. The present study was divided into three experiments according to period of treatment, first experiment undertaken to investigate the effect of colostrums through 7 days of treatment. The second experiment undertaken to investigate the effect of virgin and multipara camel milk through 30 days of treatment. Third experiment was undertaken to investigate the effect of virgin and multipara camel milk through 60 days of treatment. The results of experiments revealed that the diabetic male rats in second group suffering from significant decrease at ($p \leq 0.05$) in sperm count and sperm deformity.

Key words: Colostrums, camel milk, sperm count and diabetic rats.

1. Introduction

Diabetes mellitus is a chronic systemic disease characterized by an increased blood glucose concentration. The word diabetes is derived from the Greek word "diabainein" and means "to pass through", referring to the large volume of urine, while mellitus comes from the Latin term "mel", which means honey and refers to the sweetness of the urine from patients with untreated diabetes. Diabetes is caused by either decreased production of insulin from the pancreatic β-cells or decreased effect of insulin on target tissues or by a combination of these two. Diabetes not only causes disturbances in carbohydrate metabolism, but also affects lipid and protein metabolisms he two major categories of diabetes are type 1 and type 2 diabetes, previously also called "Insulin-dependent" (IDDM) and "Non-insulin dependent" (NIDDM) or "Juvenile" and "Adult-onset" diabetes, respectively. Type 1 diabetes was characterized by an autoimmune reaction that leads to a total loss of function of the insulin - secreting β-cells of the islets of Langerhans in the pancreas, resulting in absolute insulin deficiency. Type 2 diabetes is the consequence of decreased insulin sensitivity (primarily in skeletal muscles, adipose tissue, and liver) and decreased insulin secretion from β-cells. It is the most common form of diabetes and is increasing in epidemic proportions worldwide.

Considerable overlap exists between the two conditions, and type 1 and type 2 diabetes have been proposed to be different forms of the same disease, the main difference being the absence of an immune response in patients with type 2 diabetes, leading to a slower rate of β -cell loss. On the other hand, the clear lack of evidence for similar genetic factors predisposing to type 1

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and type 2 diabetes supports the notion of two separate diseases. It is predicted that about 366 million people worldwide will be diabetic by the year 2030. There are 2 types of diabetes; T1D and Type 2 Diabetes (T2D). T1D is a heterogeneous disorder associated with the destruction of pancreatic beta cells, with the resultant effect of absolute insulin deficiency. Type 2 diabetes on the other hand was characterized by resistance to insulin action and suboptimal insulin secretary response. Causes of diabetes ranges from autoimmune - mediated destruction of beta cells and idiopathic destruction or failure of beta cells. About 5 – 10 % of the total cases of diabetes worldwide are due to T1D. The T1D is the most common type of diabetes in children and adolescents while Type 2 Diabetes (T2D) is common among young adults. Type - 1 Diabetes (T1D) has been increasing by 2 % to 5 % worldwide.

2. Material and Methods

The study includes three experiments and one hundred twenty male rats have been used.

Experiment one

Effect of the treatment of camals colostrum for 7 days. Twenty four male rats were divided equally and randomly in to four groups

Group - 1: Standard normal control group consists of 6 males rats treated orally with 2 ml of normal saline for 7 days.

Group - 2: Diabetic control group consists of 6 males rats that were injected intra peritoneal (I.P.) with (150 mg/kg) dissolved in ½ ml of alloxan for induction diabetes.

Group – 3: Diabetic – insulin group consists of 6 induced diabetic rats treated with I.P. injection of (6 units /kg/day) insulin.

Group – 4: Diabetic – colostrum group consists of 6 induced diabetic rats treated orally with 2 ml/day from camels colostrum from the first age group of she camel 4 - 6 years for 7 days.

Experiment two:

Effect of the treatment with virgin and multipara milk for 30 days. Forty two male rats were divided equally and randomly in to seven groups.

Group – 1: Standard normal control group consists of 6 males rats treated orally with 2 ml of normal saline for 30 days.

Group – 2: Diabetic control group consists of 6 males rats that were injected intra peritoneal (I.P.) with (150 mg/kg) dissolved in ½ ml of alloxan for induction diabetes.

Group – 3: Diabetic – insulin group consists of 6 induced diabetic rats treated with i.p injection of (6 units /kg/day) insulin.

Group – 4: Diabetic – virgin camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the virgin camel milk for 30 days.

Group – 5: Diabetic – multipara camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the multipara camel milk for 30 days.

Group – 6: Virgin camel milk group consists of (6 rats) treated orally with 2 ml/day from the virgin camel milk for 30 days.

Group – 7: Multipara camel milk group consists of (6 rats) treated orally with 2 ml/day from the multipara camel milk for 30 days.

Experiment three:

Effect of the treatment with virgin and multipara camel milk was studied for 60 days. Fifty four male rats were divided equally and randomly in to eight groups.

Group - 1: Standard normal control group consists of 6 males rats treated orally with 2 ml of normal saline for 60 days.

Group – 2: Diabetic control group consists of 6 males rats that were injected intra peritoneal (I.P.)

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with (150 mg/kg) dissolved in ½ ml of alloxan for induction diabetes.

Group – 3: Diabetic – insulin group consists of (6 induced diabetic rats) treated with i.p injection of (6 units /kg/day) insulin.

Group – 4: Diabetic – virgin camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the virgin camel milk for 60 days.

Group - 5: Diabetic – multipara camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the multipara camel milk for 60 days.

Group - 6: Diabetic – virgin camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the virgin camel milk for 60 days, killed after 30 days from stopping treatment.

Group - 7: Diabetic – multipara camel milk group consists of (6 induced diabetic rats) treated orally with 2 ml/day from the multipara camel milk for 60 days, killed after 30 days from stopping treatment.

Group – 8: Virgin camel milk group consists of (6 rats) treated orally with 2 ml/day from the virgin camel milk for 60 days.

Group – 9: Multipara camel milk group consists of (6 rats) treated orally with 2 ml/day from the multipara camel milk for 60 days.

Seminal Analysis

Sperm Concentration

The sperms were counted by using Neubauer hemocytometer chamber which use for RBC and WBC count according to method of Robb *et al*. (1978). The epididymis were put in a petridish contained 5 ml of 0.9 % normal saline. The epididymis was cut into 6 – 10 pieces by using sharp scalpel. The suspension resulted from the previous step was filtered by clean piece of gauze into a test tube. One drop from the filtrate was dropped on the Neubauer chamber which covered previously with cover slid. The sperms found on the five squares that use for counting the RBCS by using the objective lens (40 x).

Percentage of Abnormal Spermatozoa

The estimation of the percentage abnormal spermatozoa was done by using same slide that was used in the measurement of the dead and live spermatozoa, two hundred sperms were counted under the light microscope using 100 X power (Filler, 1993).

Statistical analysis

The statistical analysis used the software SPSS version 19.0. The result was expressed as mean ± standard error (mean ± SE). One way ANOVA was used to compare parameters in different studied groups. P-values ($P<0.05$) were considered statistically significant.

3. Result and Discussion

Effect of treatment on sperm count and sperm abnormality

Effect of colostrum on sperm count and sperm abnormality after 7 days of treatment.

The results listed in Table - 1 record a significant decrease in sperm count in diabetic group compared to all other groups, whereas 4th group increase significantly compared to 3rd group while there were not compared to normal group. The Table - 1 all so showed the of sperm abnormality indicated that diabetic group increase significantly at ($p < 0.05$) in comparison with all other group, while 3rd and 4th group decrease significantly compared to diabetic group but there were no significantly between them, whereas the same tow group increase significantly in comparison with normal control.

Effect of virgin and multipara she camel milk treatment other sperm count and sperm a abnormality after 30 days of treatment

As seen in Table – 2, the sperm count revealed a significant decrease in 2nd and 3rd

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Table – 1: Effect of 7 days treatment of colostrum she camel on sperm count and sperm abnormality of control and experimental groups of male rats

Groups	Sperm count Mean± S.D	Sperm abnormality Mean± S.D
Normal control group treated with 2 ml DW\ day for 7 days	56.33 ± 1.75	21.71 ± 1.57
Diabetic control group (7 days)	42.16 ± 2.85	55.05 ± 3.02
Diabetic group treated with insulin (7 days)	48.16 ± 1.32	36.16 ± 2.78
Diabetic group treated with colostrum (7 days)	53.50 ± 3.93	31.83 ± 2.71
LSD	5.33	4.33

Table - 2: Effect of 30 days treatment of virgin and multipara she camel milk on sperm count and sperm deformity of control and experimental groups of male rats

Groups	Sperm count Mean± S.D	Sperm abnormalities Mean± S.D
Normal control group treated with 2 ml DW\day (30 days)	59.66 ± 3.66	21.00 ± 1.41
Diabetic control group (30 days)	33.50 ± 1.04	65.5 ± 3.14
Diabetic group treated with insulin (30 days)	31.50 ± 1.37	41.00 ± 3.52
Diabetic group treated with virgin she camel milk (30 days)	134.16 ± 48.37	31.50 ± 2.42
Diabetic group treated with multipara she camel milk (30 days)	83.00 ± 9.33	33.66 ± 2.25
Standard control group treated with virgin she camel milk (30 days)	117.33 ± 24.70	21.33 ± 1.21
Standard control group treated with multipara she camel milk (30 days)	90.66 ± 3.38	23.66 ± 1.366
LSD	26.16	3.00

Table – 3: Effect of 60 days treatment of virgin and multipara she camel milk on sperm count and sperm deformity of control and experimental groups of male rats

Groups	Sperm count Mean± S.D	Sperm abnormalities Mean± S.D
Normal control group treated with 2 ml DW\day (60 days)	59.33 ± 3.66	21.83 ± 1.60
Diabetic control group (60 days)	29.50 ± 1.04	62.00 ± 4.42
Diabetic group treated with insulin (60 days)	32.50 ± 1.37	35.66 ± 3.38
Diabetic group treated with virgin she camel milk (60 days)	91.50 ± 9.56	23.33 ± 3.38
Diabetic group treated with multipara she camel milk (60 days)	81.00 ± 4.89	31.66 ± 1.36
Diabetic group after one month form stopping 60 days treatment with virgin she camel milk	73.33 ± 5.35	38.33 ± 2.25
Diabetic group after one month form stopping 60 days treatment with multipara she camel milk	64.50 ± 3.33	44.00 ± 2.28
Standard control group treated with virgin she camel milk (60 days)	117.33 ± 24.70	21.33 ± 1.21
Standard control group treated with mulipara she camel milk (60 days)	90.66 ± 3.38	23.66 ± 1.36
LSD	14.00	4.00

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groups compared to other groups and they showed non-significant differences between them. On the other hand the 4th and 6th group record a significant increase compared to other groups, while 5th and 7th group increase non significantly compared to normal control. Depending on the results listed in Table - 2, there were a significant increase at ($p < 0.05$) in sperm a abnormality in diabetic group compared to all other groups, while 6th and 7th group showed non-significant difference compared to normal control and decrease significantly compared to other groups. There were non-significant difference between the 4th and 5th groups, while there were a significant decrease in 4th and 5th groups in comparison to the 3rd group.

Effect of virgin and multipara she camel milk treatment on the sperm count and sperm a abnormality during 30 days of treatment

The result in the Table - 3 indicate that the diabetic group and 2nd group showed a significant decrease ($p<0.05$) in sperm count compared to other group but there were no any significantly between them, while 4th, 5th, 6th and 9th group increase significantly compared to normal control and there were non-significant difference between them, whereas 7th group revealed non-significant difference in comparison with 7st group. On the other hand, the 8th group showed a significant increase compared to other all groups.

The Table - 3 clarified the sperm a abnormalities after 60 days of treatment, diabetic group showed significant increase in sperm abnormality in comparison with other groups while there were no significant difference between the 4th, 8th and 9th group compared to normal group and between them. on the other hand the 3rd and 6th group decrease significantly at ($p < 0.05$) compared to 2nd group but there were non-significant difference between then. Whereas, the 7th group increase significantly compared to other group while decrease significantly compared to diabetic group.

The rats treated with alloxan to induced diabetes show a significant decrease in the number of sperm compared with central group as a result of testicular tissue changes result from the high level of oxidative stress. Thus, it causes lipid peroxidation of fat intesticalar tissue result from increase formation of free radicals (Emanuelle *et al*., 1991; Husain and Somani, 1997). Sex hormone and disruption result in imbalance in the endocrine and this will lead to a decline in the number of spermatogonia (Jelodar *et al*., 2009; Kim *et al*., 2014).

The increased lipid per oxidation lead to increase the alteration of sperm membrane function impair development of sperm and reduced it is motility and also oxidative damage to sperm DNA (Aitken *et al*., 1989; Julie, 2003). Rates treated with colostrum and CM show improve in semen characteristics properties because they contains several antioxidant vitamins in high concentration like vitamin C, E, B2 and A and highly rich in trace elements e.g., zinc and magnesium (Yousef, 2006). The vitamin E act as free radical scavenger and anti oxidant molecules beside it is necessary for normal activity of oxidative enzyme. The vit C scavengers superoxide, H_2O_2 and hydroxyl radicals so it prevent sperm agglutination, and as well as prevent lipid per oxidation and protect against DNA damage induced by H_2O_2 radical (Gu rney *et al*., 1996; Veldink, 2007), while magnesium helps in the absorption and metabolism of different vit (E, B, C) (Barbagallo *et al*., 2009). Zinc found in large quantity in camel milk (Ozdemir and Inanc, 2005; Yonsef, 2006), that can block cellular deterioration by antioxidant system activation (Zhiguo *et al*., 2012). Sperm cell are metabolically active and generate large number of free radicals during their development neutralize by zinc thereby improving sperm quality (Haas, 2006; Colagar *et al*., 2009).

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