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Role of Camel Milk and its Derivatives in Maintaining Lipoprotein Level in Alloxan-Induced Diabetic Rabbits

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ABSTRACT: This study was designed to explore the role of camel milk, colostrums and its derivatives in maintaining lipoprotein level in Alloxan-Induced diabetic rabbits. For study purpose, thirty clinically normal rabbits of both sexes closely in weight and age were used. Completely Randomized Design (CRD) was used where they were divided to six groups each with five rabbits. The animals were fed with green carrot (Caucus Carrot) and tap water and provided with air-conditioned quarters at 24°C under standard husbandry conditions. Diabetes in the rabbits was induced by intravenous injection of Alloxan. Fresh solution of Alloxan was prepared and the rabbits in six groups were administered by 80 mg/Kg body weight of the solution while one group was left untreated with Alloxan as a control group. The diabetic rabbits were then treated with fresh camel milk, fermented camel milk (gars) and colostrums as well as insulin for 60 days. The results deduced that treatments of diabetic rabbits with colostrums, camel milk, gars and insulin resulted in decreasing the high-density lipoprotein (HDL) levels from 41.9 to 9 mg/dl, but it kept within the normal and permissible levels of 40-60 mg/dl. The diabetic rabbits treated with colostrums, camel milk and insulin resulted in decreasing the Very Low-Density Lipoprotein (VLDL) levels, but kept within the normal levels and maintained at desirable level (less than 35 mg/dl). While treated with gars was resulted in a higher and not desirable VLDL level. Key words: Lipoprotein level, HDL, VLDL, Alloxan, Diabetic rabbits

INTRODUCTION

The diet rich in saturated fats, smoking, lifestyle, and increasing in visceral fat is raising the Low Density Lipoprotein (LDL) cholesterol level [1]. Significant higher level of triglycerides in Sudanese diabetic patients may due to overproduction of VLDL lead to increased plasma levels of triglyceride which, via an exchange process mediated by cholesterol ester transfer protein (CETP), result in lower levels of high density lipoprotein HDL-cholesterol, which results faulty glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue resulting in hyperglycemia. The fatty acids from adipose tissue are mobilized for

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energy purpose and excess fatty acid is accumulated in the liver, which are converted to triglyceride [2].

Elevated levels of cholesterol in the blood are regarded as a major risk factor for heart disease. It has been demonstrated that, administration of fermented camel milk has a hypo-cholesterolemic effect in rats [3]. Hypo-cholesterol mechanism of camel milk is still unclear, but different hypotheses were discussed, including: the interaction between bioactive peptides from camel milk and cholesterol levels is derived, which lead to cholesterol-lowering [4] and the presence of orotic acid in camel milk (arises as an intermediate in the metabolism of the nucleic acids), which is considered responsible for the lowering of cholesterol levels in rats [5] and in humans [6]. [7] reported that data concerning the management of high triglyceride (TG) levels and low HDL cholesterol levels remains inconclusive. So this study was intended to explore the role of camel milk, colostrums and its derivatives in maintaining lipoprotein level in Alloxan-Induced diabetic rabbits.

MATERIALS AND METHOD

Materials

Water bath (37°C), Analyzer, Spectrophotometer, Cuvette at 37°C for readings at (main wavelength) 600 \pm 20 nm and (secondary wavelength) 700 nm \pm 20 nm, Desktop centrifuge and Thermostatic water bath at 37°C.

Method

Experimental design

Thirty clinically normal rabbits of both sexes closely in weight (3-3. 5 Kg) and age (one ear old) were provided; Completely Randomized Design (CRD) was used where they were divided to six groups each with five rabbits. The animals were fed with green carrot (*Caucus Carrot*) and tap water and provided with air-conditioned quarters at 24°C under standard husbandry conditions.

Alloxan inducing diabetes

Diabetes to rabbits was induced by intravenous injection of Alloxan [8]. Fresh solution of Alloxan was prepared and the rabbits in six groups were administered by 80 mg/Kg body weight of the solution while one group was left untreated with Alloxan as a control group. After a week of Alloxan injection, diabetes was confirmed through the measurement of blood glucose levels from heart blood using glucometer (Prestige). Rabbits with blood glucose concentration \geq 8.0 mmol / L were selected for the experiment.

Treatments groups

The treated groups were designated as follow:

i. Group 1 (control) to which no Alloxan induction no Fresh camel milk, colostrums and fermented camel milk supplementation.

ii. Group 2 (diabetic-non supplemented) to which diabetes was induced but no Fresh camel milk, colostrums and fermented camel milk supplementation.

iii. Group 3 (diabetic-treated) to which diabetes was induced and supplement with fresh camel milk, each rabbits in Group 3 was daily treated with 5ml of camel milk using 5ml syringe for oral administration for 4 weeks, and the dose was then increased to 5ml for additional 8 weeks.

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iv. Group 4 (diabetic-treated) to which diabetes was induced and supplemented with5ml of Colostrums, each rabbits in Group 4 was treated daily with Colostrums using 5ml syringe for oral administration for 4 weeks where the dose was then increased to 5ml for additional 8 weeks.

v. Group 5 (diabetic-treated) to which diabetes was induced and supplemented with5ml of fermented camel milk, each rabbits in Group 5 was daily treated with Gars using 5ml syringe for oral administration for 4 weeks and the dose was then increased to 5ml for additional 8 weeks.

vi. Group 6 (diabetic-treated) to which diabetes was induced and supplemented with Insulin and each rabbits in Group 6 was daily treated with Insulin by injection (16 mg/kg body wt) for 12 weeks.

Determination of high-density lipoprotein

Reagents were Preheat at 37°C for a few minutes, Pipette was placed into a bucket and mixed and inserted into the thermostatted cuvette holder at 37°C. After 5 minutes, absorbance (A1) at 600/700 nm was read against distilled water. Pipette was then placed into the cuvette. After 5 minutes absorbance (A2) at 600/700 nm was then read. The concentration of high density lipoprotein (HDL) cholesterol was calculated from the following general formula:

Determination low density lipoprotein

Pipette was put into labeled centrifuge tubes and mixing thoroughly and let stand for 15 minutes at room temperature. Centrifugation was conducted at a minimum of 4000 r.p.m. for 15 minutes. Supernatant was collected. The reagent (Cholesterol kit) was kept to room temperature. Pipette was put into labeled test tubes. Mixing was thoroughly conducted and the tubes were incubated for 30 minutes at room temperature (16-25°C) or for 10 minutes at 37°C. The absorbance (A) of the standard and sample at 500 nm against the blank was measured and the color was kept stable for 30 minutes. Low density lipoprotein (LDL) cholesterol concentration in the supernatant was calculated using the following formula:

Determination of very low-density lipoprotein

Pipette was put in labeled centrifuge tubes. Mix was done thoroughly and let stand for 10 minutes at room temperature. Centrifugation was carried out at a 4000 r.p.m. for 10 minutes and supernatant was then collected. The reagent (Cholesterol kit) was brought to room temperature. Pipette was put into labeled test tubes. Very low-density lipoprotein (VLDL) cholesterol concentration in the supernatant was calculated using the following general formula:

Statistical analysis

Results were represented as means and standard deviation of three replicates. The variance was analyzed and the differences between means were evaluated using Duncan's Multiple Range student t-test at 0.05 level of significance.

RESULTS AND DISCUSSION

Effect of camel milk and derivatives on high density lipoprotein

Table (1) demonstrated that in case of Group 1 (non diabetic- none supplemented), the HDL levels were fluctuating between 41.9 to 44.2 mg/dl throughout the experimental period from 0- 60 days and found to be within normal range of 40- 60 mg/dl. In case of Group 2 (diabetic-non supplemented) the level of HDL was decreased from 45.0 mg/dl at 0 days to reach level of 30.4 mg/dl at 60 days as the lowest value as compared with other groups. From 0 day to 60 days, in Group 3 (diabetic-treated with

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colostrums) the HDL level increased from 43.0 mg/dl to 63.1 mg/dl, in Group 4 (diabetic-treated with milk) increased from 41.8 mg/dl to 54.4 mg/dl, in Group 5 (diabetic-treated with gars) from 43.4 mg/dl to 45.3 mg/dl and in Group 6 (diabetic-treated with Insulin16 mg/kg body weight daily for 8 weeks) was increased from 42.1 mg/dl to 66.6 mg/dl. It was demonstrated that there were no significant differences ($P \ge 0.05$) in the HDL levels between Group 1, Group 3, Group 4, Group 5 and Group 6 at 0.05 level of significance, and the values were found to be within the normal range of 40- 60 mg/dl. The lowest level of total HDL at 60 days was 30.4 mg/dl and recorded by Group 2 (diabetic-treated with Insulin16 mg/kg of body weight daily for 8 weeks) showed level of HDL of 62.1 mg/dl and 66.6 mg/dl respectively which were at desirable level (higher than 60 mg/dl). It is concluded that treatments of diabetic rabbits with colostrums, camel milk, gars and insulin resulted in increasing the HDL levels, but kept within the normal levels and the treatment with colostrums or Insulin achieved desirable level of HDL. Diabetes mellitus reduced the level of HDL if no treatment was applied as shown in case of Group 2. The present result was found to be agreed with [9] finding that camel milk raised the High-Density Lipoprotein level.

 Table 1. Effect of fresh and fermented camel milk and colostrums on high density lipoprotein level in diabetic rabbits

0 day	15 days	30 days	45 days	60 days		
41.9 ± 1.9^{a}	44.2±1.5a	45.0±1.2a	44.1±1.2a	43.1±1.0a		
$45.0{\pm}1.1^{a}$	41.0±1.3a	38.1±1.7b	34.6±1.5b	30.4±1.8b		
$43.0{\pm}1.4^{a}$	47.0±1.2a	52.2±2.0c	57.7±1.3cd	62.1±2.0c		
41.8 ± 1.6^{a}	43.0±1.3a	47.0±2.3a	51.3±1.1c	54.4±1.5d		
43.4 ± 0.7^{a}	42.7±1.0a	44.1±1.2a	43.2±1.0a	45.3±0.8a		
42.1 ± 1.3^{a}	47.4±1.6a	52.2±1.4c	59.0±1.7d	66.6±1.4c		
	$\begin{array}{c} 41.9 \pm 1.9^{a} \\ 45.0 \pm 1.1^{a} \\ 43.0 \pm 1.4^{a} \\ 41.8 \pm 1.6^{a} \\ 43.4 \pm 0.7^{a} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$		

*Each value is mean \pm SD of four replicates.

*Values in column share same superscript letter show no significant difference at p = 0.05 as separated by Duncan's Multiple Test.

* Normal range 40 - 60 mg/dL or desirable (higher than 60).

Effect of camel milk and derivatives on low density lipoprotein

Table (2) showed that in Group 1 (non diabetic - none supplemented) the Low Density Lipoprotein (LDL) levels was fluctuating between 71.8 to 73.4 mg/dl throughout the experimental period from 0-60 days and found to be within normal range of 100- 129 mg/dl. In case of Group 2 (diabetic- non supplemented) the level of LDL was increased from 70.0 mg/dl at 0 days to reach the maximum level of 147.1 mg/dl at 60 days as the highest value as compared with other groups and higher than normal and permissible level. From 0 day to 60 days, in Group 3 (diabetic-treated with colostrums) LDL levels were decreased from 92.3 mg/dl to 75.4 mg/dl, in Group 4 (diabetic-treated with milk) decreased from 78.1 mg/dl to 69.6 mg/dl, in Group 5 (diabetic-treated with gars) from 73.4 mg/dl to 70.1 mg/dl and in Group 6 (diabetic-treated with Insulin16 mg/kg body weight daily for 8 weeks) was decreased from 77.3 mg/dl to 67.4 mg/dl. It was demonstrated that there were no significant differences at 0.05 in the LDL levels between Group 1, Group 3, Group 4, Group 5 and Group 6 at 0.05 level of significance, and the values were found to be within the normal range of 100- 129 mg/dl, and at permissible level (less than 100). It is deduced that treatments of diabetic rabbits with colostrums, camel milk, gars and insulin resulted in decreasing the LDL levels, but kept within the normal and permissible levels. The

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findings agreed with [10] conclusion that, camel milk showed a hypolipidemic effects in rats. The current result was found to be on line with [9] that camel milk reduced the low Density Lipoprotein.

 Table 2. Effect of fresh and fermented camel milk and colostrums on low density lipoprotein

 level in diabetic rabbits

Groups	0 day	15 days	30 days	45 days	60 days
Group 1	72.3±2.8 ^a	73.4±2.0 ^a	71.8±1.4 ^a	72.2±2.0 ^a	$72.0{\pm}1.6^{a}$
Group 2	$70.0{\pm}2.5^{a}$	94.7 ± 1.5^{b}	118.6 ± 2.4^{b}	129.3 ± 1.3^{b}	147.1 ± 2.2^{b}
Group 3	75.3±2.1 ^a	$90.0{\pm}1.8^{b}$	89.9 ± 1.4^{c}	80.6 ± 2.2^{c}	73.4 ± 2.5^{a}
Group 4	74.1 ± 1.2^{a}	76.5 ± 1.5^{a}	$75.0{\pm}1.1^{a}$	$73.4{\pm}1.4^{a}$	69.9 ± 1.6^{a}
Group 5	73.4±1.1 ^a	$71.0{\pm}1.3^{a}$	70.3 ± 1.4^{a}	$71.0{\pm}1.0^{a}$	70.1 ± 1.3^{a}
Group 6	75.3 ± 2.0^{a}	$75.0{\pm}1.5^{a}$	72.5 ± 1.3^{a}	$70.0{\pm}1.5^{a}$	67.4 ± 1.2^{a}

*Each value is mean \pm SD of four replicates.

*Values in column share same superscript letter show no significant difference at p = 0.05 as separated by Duncan's Multiple Test.

* Normal range 100 - 129 mg/dL or desirable (less than 100).

Effect of camel milk and derivatives on very low-density lipoprotein

In Table (3) Group 1 (non diabetic-none supplemented) showed fluctuation in the Very Low Density Lipoprotein (LDL) levels between 28.53 to 29.08 mg/dl throughout the experimental period from 0-60 days, and found to be within normal range (5-35 mg/dl), and at desirable limit (less than 35 mg/dl). In case of Group 2 (diabetic-non supplemented) level of Very Low-Density Lipoprotein (VLDL) was increased from 47.8 mg/dl at 0 days to reach the maximum level of 67.7 mg/dl at 60 days as the highest value as compared with other groups and the values were higher than normal range. From 0 day to 60 days, in Group 3 (diabetic-treated with colostrums) the VLDL level was decreased from 45.8 mg/dl to 33.0 mg/dl, in Group 4 (diabetic-treated with milk), and decreased from 45.4 mg / dl to 34.6 mg/dl, in Group 5 (diabetic-treated with gars), also decreased from 41.6 mg/dl to 35.8 mg/dl which remained higher than normal range (5-35 mg/dl), and in Group 6 (diabetic-treated with Insulin16 mg/kg body weight daily for 8 weeks) the VLDL level was decreased from 44.8 mg/dl to 30.8 mg/dl. It was demonstrated that there were no significant differences at 0.05 in the VLDL levels between Group 1, Group 3, Group 4, Group 5 and Group 6 at 0.05 level of significance, except group 5 the values were found to be higher than the upper limit of the normal range of 5- 35 mg/dl. The highest level of VLDL at 60 days was recorded by Group 2 (diabetic-non supplemented). It is deduced that treatments of diabetic rabbits with colostrums, camel milk and insulin as shown in Group 3, Group 4 and Group 6 respectively was resulted in decreasing the VLDL levels, but kept within the normal levels and maintained at desirable level (less than 35 mg/dl). While treatment with gars as recorded by Group 5, resulted in a higher and not desirable of VLDL level. The results were on line with Kamal et al (2018) conclusion that camel milk reduced the VLDL.

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Table 3. Effect of fresh and fermented camel milk and colostrums on very low density lipoprotein level in diabetic rabbits

Groups	0 day	15 days	30 days	45 days	60 days
Group 1	28.60 ± 1.3^{a}	28.53 ± 1.0^{a}	29.08±1.2 ^a	28.66 ± 1.0^{a}	28.90 ± 1.4^{a}
Group 2	47.80 ± 1.5^{b}	53.20 ± 1.6^{b}	57.80 ± 1.3^{b}	64.60 ± 1.5^{b}	67.70 ± 2.0^{b}
Group 3	45.80 ± 1.2^{b}	$41.60 \pm 1.6^{\circ}$	$37.40 \pm 1.4^{\circ}$	$34.00 \pm 2.0^{\circ}$	$33.00 \pm 1.6^{\circ}$
Group 4	45.40 ± 1.1^{b}	$42.00 \pm 1.5^{\circ}$	$38.14 \pm 1.5^{\circ}$	$36.00 \pm 1.4^{\circ}$	$34.60 \pm 1.8^{\circ}$
Group 5	44.60±0.9 ^b	$40.00 \pm 1.3^{\circ}$	38.20±1.7°	$36.40 \pm 1.5^{\circ}$	$34.92 \pm 1.6^{\circ}$
Group 6	44.80±2.0 ^b	$40.20 \pm 1.4^{\circ}$	36.00±2.1°	32.80±1.3°	$30.80 \pm 1.5^{\circ}$

*Each value is mean \pm SD of four replicates.

*Values in column share same superscript letter show no significant difference at p = 0.05 as separated by Duncan's Multiple Test.

* Normal range 5 - 35 mg/dL or desirable (less than35).

CONCLUSION

Applying colostrums, camel milk, gars and insulin in diabetic rabbits resulted in increasing the High Density Lipoprotein levels (HDL). On the other hand treatment with colostrums or Insulin resulted in desirable level of the High Density Lipoprotein (HDL). Diabetes mellitus reduced the level of High Density Lipoprotein if no treatment was applied. Treatments of diabetic rabbits with colostrums, camel milk, gars and insulin resulted in decreasing the Low Density Lipoprotein levels (LDL). While treatment with gars in some case, resulted in a higher and not desirable level of Very Low Density Lipoprotein (VLDL).

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