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EFFECT OF THYMOQUINONE AND CAMEL MILK ON LIVER FUNCTION OF STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

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ABSTRACT
The present study was designed to evaluate the beneficial hepatoprotective effects of thymoquinone (TQ) and camel milk (CM) on streptozotocin induced diabetic albino rats by assaying liver function enzymes (ALT&AST) and serum protein profile in both prophylactic and treated groups. The serum AST showing a significant decrease in its activity in all groups after treatment ,while CM in prophylactic group has the most significant effect followed by TQ in treated group. However, TQ in treated group exhibited the most significant effect on ALT activity along with the same significant decrease in the other groups. Given that the high protein content of camel milk, total proteins and globulin in both CM prophylactic and treated groups were significantly improved more than TQ prophylactic and treated groups. Otherwise, albumin concentration improved significantly in all tested groups. Moreover, highly significant improvement in A/G ratio of both TQ prophylactic and treated groups more than those of CM prophylactic and treated groups. In conclusion, administration of both thymoquinone and camel milk exhibited ameliorative and hepatoprotective beneficial effects on diabetic albino rats.

KEYWORDS: Thymoquinone, camel milk, hepatoprotective, prophylactic, treated groups.

INTRODUCTION
Diabetes mellitus is a global metabolic disorder of the metabolism of carbohydrates, lipids and proteins which is characterized by a high fasting blood sugar, which caused serious endocrine disorder that causes millions of deaths worldwide.[1][2] The putative role of the liver in the pathogenesis of type 2 diabetes mellitus has gained much interest and several cross-sectional and prospective studies have shown associations of ALT with type 2 diabetes mellitus and the metabolic syndrome. Hanley and co-workers studied the relation of four different liver enzymes (including ALT) with the development of the metabolic syndrome in a multiethnic cohort and demonstrated that ALT was associated with an increased risk of incident metabolic syndrome.[3] In a prospective study, serum ALT concentrations were related to both hepatic insulin resistance and later decline in hepatic insulin sensitivity. Moreover, Vozarova et al. suggested that a raised ALT reflects fatty changes in the liver and that this abnormality antedates the development of type 2diabetes[4] and addition several studies confirmed these reports.[5][6][7] However, different population-based studies could not demonstrate independent associations of ALT with risk of type 2 diabetes mellitus.[8] Several studies reported increase in liver transaminases activity and decrease in protein contents in diabetic rats[9][10],[11], which ameliorated significantly after treatment with Salvia officinalis, Agaricus bisporus, Pleurotus ostreatus, Medicago sativa and garlic oil.[12],[13] Moreover, diabetic rats showed significantly reduced albumin and albumin/globulin ratio compared to non-diabetic control rats.[14] Previous reports show that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin, which may be responsible for the decreased level of plasma proteins in diabetic rats.[15]

Nigella sativa or black seed is one of the medicinal plants with antihyperglycemia and antihyperlipidemia characteristics.[16][17] Nigella sativa has many different chemical ingredients including Thymoquinone (TQ) which conceder the most prominent constituent of Nigella sativa seeds oil. Furthermore, it has evidently proved its activity as hepatoprotective, anti-inflammatory, antioxidant, cytotoxic and anticancer, with specific mechanisms of action, which support to consider this compound as an emerging natural drug.[18]

Camel milk (CM) is opaque white in color with normal odor and salty taste. Camel's milk contains less casein proportion than cows' milk, rich in whey proteins and the microflora is less efficient.[19] Moreover, camel milk has insulin like activity, regulatory and immunomodulatory functions on β cells.[20] Also, camel milk exhibits hypoglycemic effect when given as an adjunctive therapy, which might be due to presence of insulin/insulin like protein in it and possesses beneficial effect in the treatment of diabetic patients.[21] The present study aimed to evaluate the treatable and hepatoprotective effects of TQ and CM on streptozotocin induced diabetic albino rats by assaying serum transaminases and protein profile in both prophylactic and treated groups.

MATERIALS AND METHODS

MATERIALS

Experimental Animals

White male albino rats (Rattus norvegicus) weighing about 120-180g were used as experimental animals in the present investigation. They were supplied from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for 10 days before the onset of the experiment to exclude any inter current infection. The chosen animals were housed in metal (stainless steel) cages at normal atmospheric temperature (25±5^{0}C) as well as 12 hrs daily normal light periods. All the procedures were performed in accordance with the Institutional Animal Ethics Committee in Beni-Suef University recommendations.

Thymoquinone and Camel milk

Thymoquinone (TQ) is a compound derived from the black seeds of a Middle Eastern flower called Nigella sativa.

The TQ was purchased from Sigma (Sigma-Aldrich Co., St. Louis, Missouri, USA), used for emulsions preparation. Oil-in-water nanoemulsion from thymoquinone was prepared by homogenizing 5% of TQ with 95% aqueous phase (5% Tween-20 and 90% double distilled-water)[22] injected daily in a dose of 50 mg/kg b.wt.

Camel milk (CM) is opaque white in color with normal odor and salty taste. It was administered in a dose of 33 ml/kg body weight for each rat daily by oral cannula.[23] Camel milk samples were collected from a Camel farm in Marsa Matrouh, Western desert, Egypt. All lactating camels consumed the same type of food. The milk was collected in the morning in sterile screw bottles and kept on ice during transportation to the laboratory where milk bottles were stored at 4°C.

METHODS

Induction of Diabetes

Treated groups

Diabetes mellitus was experimentally induced in animals fasted for 16 hrs by intra-peritoneal injection of 45 mg/kg b.wt. streptozotocin purchased from Sigma (Sigma-Aldrich Co., St. Louis, Missouri, USA) dissolved in citrate buffer, pH 4.5.[24] Ten days after streptozotocin injection, rats were screened for blood glucose levels. Overnight fasted (about 12 hrs) animals were given glucose (3 g/kg b.wt.) by gastric intubation. After 2hrs of oral administration, blood samples were taken from lateral tail vein, centrifuged and plasma glucose concentrations were measured. Rats having serum glucose ranging from 180-300 mg/dl (mild diabetes), after 2 hrs of glucose intake, were included in the experiment, while the others were excluded.

Prophylactic groups

These groups are differing from treated groups by administration of TQ and CM 2weeks before induction of diabetes experimentally by intra-peritoneal injection of 45 mg/kg b.wt. STZ purchased from Sigma dissolved in citrate buffer, pH 4.5.[24]

Experimental design and Animals Grouping

Rats (48 ones) were divided into 2 categories; the prophylactic groups and the treated groups and were divided into six groups.

1. **Group1:** The first group of normal animals were kept without treatments under the same laboratory condition and regarded as normal control group for the all groups.
2. **Group 2:** The second group was regarded as (diabetic control) for all groups and kept after diabetes induction without treatments under the same laboratory condition for 4 weeks.
3. **Group 3:** The third group (prophylactic TQ group) was injected the thymoquinone nanoemulsion (TQ) intra-peritoneal 1ml/kg b.wt. daily for 2 weeks before STZ injection and for another 2 weeks after diabetes induction.
4. **Group 4:** The fourth group (treated TQ group) was injected the thymoquinone nanoemulsion (TQ) intra-peritoneal 1ml/kg b.wt. daily for 2 weeks after diabetes induction.
5. **Group 5:** The fifth group (prophylactic CM group) was given Camel Milk (CM) by a dose of 33 ml/kg body weight for each rat daily by oral cannula for 2 weeks before STZ injection and for another 2 weeks after diabetes induction.
6. **Group 6:** The sixth group (treated CM group) was given Camel Milk (CM) by a dose of 33 ml/kg body weight for each rat daily by oral cannula for 2 weeks after diabetes induction.

By the end of the experimental period, normal, diabetic control, prophylactic and diabetic treated rats were sacrificed under mild diethyl ether anesthesia. Blood samples were taken and centrifuged at 3000 r.p.m. for 30 minutes. The clear, supernatant sera were quickly removed, divided into four portions for each individual animal and kept at –20^{o}C for further biochemical analysis.

Determination of serum AST and ALT

Serum aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (SGOT) and alanine aminotransferase (ALT)or glutamate pyruvate transaminase(SGPT) were determined according to kinetic method[25] using the reagent kit purchased from Human diagnostics (Germany).

Determination of serum protein profiles

Serum total protein concentration was determined according to the Biuret method[26] using the reagent kit purchased from Human diagnostics (Germany). Serum albumin concentration was determined using the reagent kit purchased from Human diagnostics (Germany) according to the bromocresol green method.[27] Serum globulin concentration and (A/G) ratio were determined according to from the following equations.

Globulin (g/dL) = Total protein (g/dL) – Albumin (g/dL)

$$(A/G)\ ratio = \frac{Albumin\ (g/dL)}{Globulin\ (g/dL)}$$

Statistical Analysis of Results

The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS 7, version 22; SPSS Inc., Chicago) was used for the statistical analysis. Comparative analysis was conducted by using the general linear models procedure (IBMSPSS). Values of P>0.05 were considered statistically non-significant, while values of P<0.05 were considered statistically significant. The results were expressed as mean ± standard deviation (SD) and values of P>0.05 were considered statistically non-significant, while values of P<0.05 were considered statistically significant.

RESULTS

Effect of Treatments on Liver Enzyme Activities

In the diabetic group, there was a significant (P<0.05) increase in serum alanine amino-transferase activity (ALT or GPT) as compared to the normal control. However, treated and prophylactic groups showed significant decreases activity in serum ALT in comparable to diabetic control. Thymoquinone treated group showed the closest level to the normal control group (Table 1& Figure 1).

On the other hand the diabetic group showed a significant (P<0.05) increase in serum aspartate amino-transferase activity (AST or GOT) in comparable to the normal control. Both treated and prophylactic groups of the experiment showed significant decreases in fasting serum AST activities when compared to diabetic control. Camel milk prophylactic group showed the nearest level to the normal control group (Table 1 & Figure 2).

Table (1): Serum ALT (GPT) and AST (GOT) of normal diabetic control, thymoquinone and camel milk prophylactic and treated groups.

Parameter / Group	ALT(U/L)	AST(U/L)
Nor-C	38.57 ± 2.44 [d]	19.29 ± 2.21 [d]
Dia-C	74.57 ± 4.69 [a]	38.00 ± 2.94 [a]
TQ-P	46.43 ± 3.95 [c]	28.86 ± 2.48 [b]
TQ-T	41.71 ± 3.77 [d]	26.29 ± 2.93 [d]
CM-P	49.71 ± 3.90 [bc]	22.00 ± 2.45 [c]
CM-T	51.86 ± 3.44 [b]	28.71 ± 1.70 [b]
F	P<0.05	P<0.05

Number of rats in each group was eight.
Data are expressed as Mean ± S.D.
Means which shared the same superscript symbol(s) are non-significantly different (P>0.05) while others significantly different (P<0.05).

1-Nor-C Normal control.
2-Dia-C Diabetic Control.
3-TQ-P Thymoquinone Prophylactic.
4-TQ-T Thymoquinone Treated.
5-CM-P Camel Milk Prophylactic.
6-CM-T Camel Milk Treated.

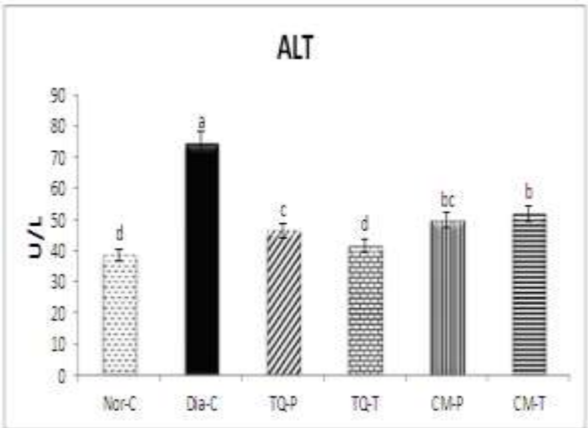

Figure (1): Serum ALT (GPT) of normal and diabetic control, thymoquinone and camel milk prophylactic and treated groups.

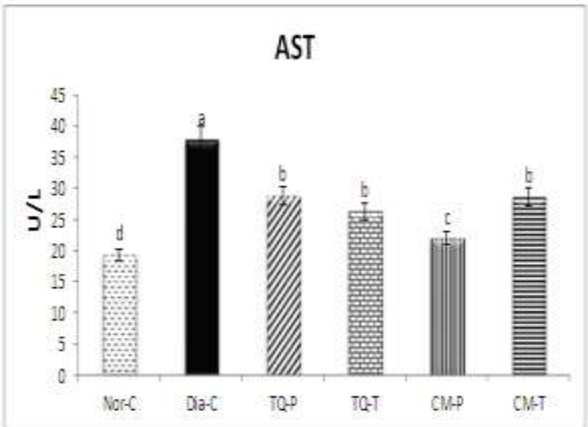

Figure (2): Serum AST (GOT) of normal, diabetic control, thymoquinone and camel milk prophylactic and treated groups.

1-Nor-C Normal control.
2-Dia-C Diabetic Control.
3-TQ-P Thymoquinone Prophylactic.
4-TQ-T Thymoquinone Treated.
5-CM-P Camel Milk Prophylactic.
6-CM-T Camel Milk Treated.

Effect of Treatments on Serum Protein profile

In the diabetic group there was a significant decrease in total proteins concentration as compared to the normal control (Table 2 and Figure 3).This decrease in serum total proteins improved significantly in all thymoquinone and camel milk prophylactic and treated groups when compared to diabetic control. Both camel milk groups showed more highly significant increase than both thymoquinone tested groups.

Otherwise, the diabetic control group exhibited significant decrease in serum albumin and globulin concentrations as compared with the that of normal control (Table 2 and Figure 4). This decrease in serum albumin improved significantly (P<0.05) in all prophylactic and treated groups when compared to untreated diabetic control. Both camel milk groups showed more highly significant (P<0.05) increase in globulin values than those of thymoquinone groups.

On the other hand, A/G ratio showed a non-significant change (P>0.05) in the diabetic group when compared with normal control group. Also, there is a non-significant increase (P>0.05) in thymoquinone prophylactic group when compared with diabetic control group. Otherwise, there were significant decreases in camel milk prophylactic and treated groups when compared with diabetic control group (Table 2).

Table (2): Serum total protein, albumin, globulin and A/G ratio of normal, diabetic control, thymoquinone and camel milk prophylactic and treated groups.

Parameter / Group	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G Ratio
Nor-C	5.91 ± 0.16 b	3.55 ± 0.09 bc	2.36 ± 0.08 bc	1.51 ± 0.03 bc
Dia-C	4.29 ± 0.36 a	2.63 ± 0.18 a	1.66 ± 0.21 a	1.60 ± 0.12 cd
TQ-P	6.41 ± 0.07 c	4.01 ± 0.11 e	2.40 ± 0.07 c	1.68 ± 0.09 de
TQ-T	5.86 ± 0.53 b	3.71 ± 0.27 cd	2.15 ± 0.34 b	1.75 ± 0.23 e
CM-P	6.60 ± 0.41 cd	3.84 ± 0.20 de	2.76 ± 0.26 d	1.40 ± 0.10 b
CM-T	6.89 ± 0.28 d	3.47 ± 0.15 b	3.41 ± 0.15 e	1.02 ± 0.03 a
F	P<0.05			

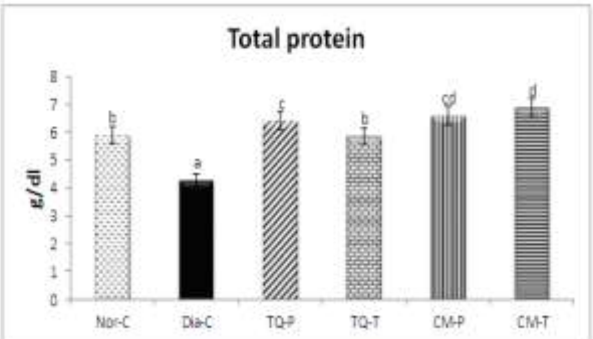

Figure (3): Serum total protein of normal, diabetic control, thymoquinone and camel milk prophylactic and treated groups.

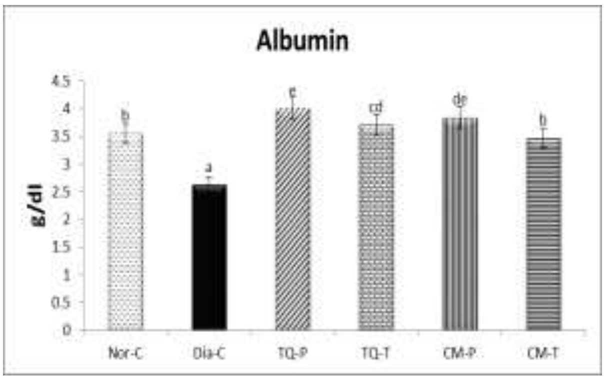

Figure (4): Serum albumin of normal, diabetic control, thymoquinone and camel milk prophylactic and treated groups.

DISCUSSION

Diabetes mellitus (DM) is characterized by impairment in insulin secretion or insulin action, which results in hyperglycemia with disturbances of carbohydrate, fat and protein metabolism.[28] Nigella sativa can improve glycemic status and lipid profile in diabetes models with various potential mechanisms including its antioxidant characteristics and effects on insulin secretion, glucose absorption, gluconeogenesis and gene expression. Some studies compared effects of various types (extract, oil, powdered) of Nigella sativa with each other and they reported different characteristics with various types of black seed.[29] Camel milk is known for its medicinal properties since ancient times and recently camel milk has been deeply studied for its special properties because of higher hepatoprotective, insulin like, antibacterial and antiviral activities.[30]

In the present study, we investigate the prophylactic and treatable hepatoprotective effects of thymoquinone (TQ) and camel milk (CM) on streptozotocin-induced diabetic albino male rats by assaying liver function test enzymes and protein profile. In diabetic group, there were significant increases in the activity of liver enzymes (ALT and AST) of diabetic rats. These results are accordance with data reported by Sunil et al. who indicated that the liver enzymes ALT and AST levels were increased in alloxan diabetic rats. This elevation reflected the generally recognized detrimental effect of hepatocyte damage, which represented in the leakage of ALT and AST from damaged hepatic cells.[31] Our results showed a significant (p<0.05) improvement in liver functions parameters (ALT and AST activities) within prophylactic and treated groups of raw camel milk. These results are in agreement with several studies[30],[32] and.[33]

Also, TQ showed the same significant ($p<0.05$) improvement in liver functions parameters appeared within prophylactic and treated groups. These results are in agreement with Bashandyet al.[34] who observed that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly decreased in the TQ treated diabetic animals as compared to diabetic control group. TQ is associated with beneficial changes in hepatic enzyme activities and thereby exerts potential antihyperglycemic effects.[35] Both Nigella sativa and TQ reduced the increased levels of serum ALT activity caused by CCl_4.[36] Further reports demonstrate that TQ protects liver dysfunction induced by aflatoxin B1 (AFB1) as evidenced by a reduction of the serum concentrations of AST and ALT as marker enzymes for liver function.[37] Nigella sativa oil administration to rats with aflatoxin-induced toxcity caused a significant ($p<0.05$) amelioration of the activities of AST and ALT.[39] Moreover, Nigella sativa oil administration showed improvement of liver function enzymes alteration induced by Malathion in human and male albino rats.[38] In addition, Nigella sativa oil administration to diabetic rats exhibited amelioration effect in serum transaninases (ALT&AST).[16]

The results of our study showed that in control diabetic group there were significant decreases in serum total proteins, albumin and globulin along with non-significant change in albumin/globulin ratio (A/G ratio). These results may be due to the structural distortion and the functional impairment of the hepatic cells which associated with low serum protein and albumin levels.[40] Our results showed that a significant ($p<0.05$) overall improvement in serum total proteins, albumin and globulin appeared within prophylactic and treated groups of camel milk restoring them near to the normal control values. Moreover, our data demonstrated significant decrease in albumin/globulin ratio (A/G ratio) in these groups. These results are in accordance to khan et al.[32] who demonstrated that camel milk feeding to diabetic rats significantly increases the content of albumin and Korish & Arafah[40] who added that is associated with the recovery of normal serum proteins and albumin levels. Additionally, in rats poisoned with Ccl_4, the level of aminotransferases, alkaline phosphatase, proteins and cholesterol values restore to almost normal levels after treatment with camel milk.[30]

Except for serum total proteins levels in TQ treated group which exhibited non-significant ($p<0.05$) changes, our results showed significant ($p<0.05$) improvement in serum total proteins, albumin and globulin appeared within TQ prophylactic and treated groups. These results are in accordance with Bashandyet al.[34], who illustrated that TQ administered to diabetic rats led to significant increase in serum total protein as compared to diabetic control rats. Abdel-Moneim et al.[39] indicated that treatment of male rats with Nigella Sativa seed extract was effective to improve the serum concentration of albumin altered by aflatoxin. Treatment with Nigella sativa seed extract has been found to limit the increase in serum albumin level induced by Schistosoma mansoni infection.[41][42] Most interestingly, supplementation of Nigella sativa oil in humans produced a significant increase in serum total proteins, albumin and globulin.[43]

Given that the high protein content of camel milk, total proteins in both CM prophylactic and treated groups naturally significantly improved more than in both TQ prophylactic and treated groups. Otherwise, albumin improved significantly in all groups and restored to normalcy. Apparently, that means CM in both prophylactic and treated groups produced significant high levels of globulin more than both TQ prophylactic and treated groups. That illustrates the highly significant improvement of A/G ratio in both TQ prophylactic and treated groups more than CM prophylactic and treated groups. Furthermore, that indicates the high hepatoprotective properties of camel milk more than thymoquinone.

The aim of the diabetes treatment is primarily to save life and alleviate symptoms and secondary to prevent long term diabetic complications and by eliminating various risk factors. There are a number of plants and natural products which have the capacity to induce the utilization of glucose and combat with secondary complications in the body organs.[44]

CONCLUSION

The above data indicated that TQ and CM prophylactic and treated groups showed obvious improvements in serum transaminases (ALT & AST) activities and protein profile in diabetic albino rats which may be due to their antioxidant antidiabetic properties.

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Nil.

Conflict of interest

None Declared.

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