Comparative Effect of Camel Milk and Black Seed Oil in Induced Diabetic Female Albino Rats

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ABSTRACT
In present study, anti-diabetic effect of camel milk and black seed oil, separate and combined, on hematobiochemical and histological parameters of selected organs was evaluated. Forty female albino rats were split into 5 groups (n=8): Group A was kept as normal. Alloxan® was administered in groups B to E for induction of diabetes. Group B was considered diabetic control while group C, D and E were offered camel milk @ 40ml/kg/day, black seed oil @ 0.8ml/day in separate and combination respectively for 30 days. Weekly blood glucose level was monitored. Animals were slaughtered to collect: blood for hematology and serum analysis and tissues for histology. Microscopy of H&E stained tissues was done at 100X to observe degenerative alterations in liver, kidneys and uterus. Least Significance Difference (LSD) test was used to compare the group means. Diabetes showed significant (P<0.05) impact on all parameters studied except uterine histometry which remained unaltered. Camel milk and black seed oil, separate or combined, significantly (P<0.05) recovered diabetic altered hematological (RBC, PLT, WBC, MCV, Hb, lymphocyte (%), MCH) and serological parameters (AST, ALT, Creatinine, BUN). Combined therapy of these agents showed more significant (P<0.01) reduction in blood glucose level as compared to their individual effects. Histological observations demonstrated that camel milk and black seed oil improved the altered histology of liver and kidneys towards normal. Hence, camel milk and black seed oil collectively have potential to restore diabetogenic hematological and serological parameters with capacity to constraint the diabetic encounter on liver and kidneys’ histology.

INTRODUCTION
Diabetes mellitus (DM) is a metabolic and endocrine disorder discriminated by elevated level of blood glucose (hyperglycemia) that results from inability of pancreas to produce plentiful insulin (DM type 1) or failure of proper response to insulin (DM type 2) (Shori, 2015; Cheng et al., 2013). It is predicted that global prevalence of diabetes in 2007 was 246 million and will rise up to 300 million by 2025 (Cheng et al., 2013; Khan et al., 2012). This dramatic change in its prevalence is due to obesity and changes in lifestyle (Baragob, 2015). In DM, reactive oxygen species are generated through a series of biochemical reactions which confound body functions (El-Saïd et al., 2010). Hyperglycemic patients have 25-50% more chances to develop renal disorders (Mir and Darzi, 2009), hepatic cirrhosis and carcinoma of hepatic cells due to altered serology (Ragavan and Krishnakumari, 2006). Earlier studies explored diabetes consequences in uterine weight loss because of atrophy of endometrium and myometrium. Diabetogenic alterations in the cellular structure of the uterus constraint its normal physiological functions and contractive response to oxytocin (Favaro et al., 2010; Housawi et al., 2015).

Natural products having antihyperglycemic activities are preferred for the control of DM because they have more safety margin for liver, kidneys and pregnancy as compared to synthetic antidiabetic drugs (Baragob, 2015). In today’s modern world, therefore, the researchers are emphasizing towards the validation of natural medicinal products with antihyperglycemic properties.

Camel milk (CM) carries many properties to optimize insulin secretion and its action. The antidiabetic activity of CM is attributed to existence of insulin like protein, ranges from 45-128 IU/liter of milk, which are coated and can endure stomach pH. CM contains minerals (sodium,
potassium, iron, copper, zinc and magnesium) especially high level of zinc, acts as co-enzyme which stimulates antioxidant system, helps in insulin secretory activity of beta cells of pancreas. It also contains vitamin C, 5 times higher as compared to other ruminants’ milk, functions as antioxidant by activating glutathione (Mullaicharam, 2014; Rahimi et al., 2011).

*Nigella sativa* or black seed (BS) has recently gained attention for its broad spectrum medicinal properties which are due to presence of chemical components like thymoquinone, flavonoids, unsaturated fatty acids, nigellone, carvone and p-cymene (Heshamati and Namazi, 2015; Wadaan, 2009). Antidiabetic property of BS is attributed to thymoquinone, a strong antioxidant, suppresses the expression of inducible nitric oxide synthase in rat macrophages (AliMohammadi et al., 2013).

Keeping in view the antidiabetic effect of these natural products, the present study was designed to evaluate the antihyperglycemic effect of CM and BS oil, separate or combined, on hematology, serology and histology of selected organs in diabetic albino rats.

**MATERIALS AND METHODS**

**Animals:** This study was carried out on a total of forty healthy adult albino rats weighing between 150 to 200g. The rats were kept for two weeks in the animal house of Institute of Microbiology, University of Agriculture, Faisalabad for adaptation under optimally maintained environmental room conditions and 12 h light/dark cycle. The rats were offered standard commercial diet and water Ad libitum. The experiment was carried out in accordance with the guidelines of the Directorate of Graduate Studies and Institutional Animal Ethical Committee.

**Therapeutic agents:** Fresh camel milk (CM) was collected daily for one month, early in the morning from a local farmer. CM was collected in sterile bottles and kept at cool place until transported to the laboratory. Prepared black seed (BS) oil was purchased from local market named as Marhaba Klonji Oil®.

**Induction of diabetes:** Diabetes was induced in 12 hour fasting rats by single intraperitoneal injection of Alloxan® @ 150 mg/kg of body weight (Shehata and Moussa, 2014). This drug damages the islet of β Langerhans on pancreas which leads to DM. The blood glucose level was measured 4 days’ post injection to ensure the induction of diabetes. Rats with fasting blood glucose >250mg/dL were considered as diabetic. The treatment was started after confirmation of diabetes for a period of 30 days.

**Experimental design:** The animals were split into 5 groups such that each group contained eight rats in the following design. Group A: Normal without diabetes induction and treatment. Group B: Diabetic control without treatment. Group C: Diabetic with oral CM treatment @ 40ml/kg/day for thirty days. Group D: Diabetic with oral BS oil treatment @ 0.8ml/day for thirty days. Group E: Diabetic with oral CM and BS oil combined treatment for thirty days (Hassan and Bayoumi, 2010; Wadaan, 2009).

**RESULTS**

Mean±SEM value of hematological parameters including red blood cells (RBCs), platelet (PLT), white blood cells (WBCs), lymphocyte (%), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in different groups of albino rats at the end of trial are given in Table 1. There was observed a significant (P<0.05) reduction in the values of these parameters in diabetic control group as compared to the normal. Camel milk (CM) and black seed (BS) oil treatment, separate or combined, significantly (P<0.05) improved hyperglycemic altered erythrocytes and its indices, leucocytes and lymphocytes but not the platelets count. A significant (P<0.05) increase in platelet count was observed in case of individual treatments.

**Sampling:** Fasting blood glucose level was monitored on weekly basis with On Call EZ H® blood glucose monitor after the induction of diabetes. Rats were slaughtered 30 days’ post treatment. Blood was collected in the vacutainer with and without anticoagulant for hematology and serum analysis respectively. Vacutainers without anticoagulant were subjected to centrifuge at 1500 rpm for 15 minutes for separation of serum. The serum was stored at -20°C for further analysis. Samples of liver, kidneys and uteri were collected and washed with normal saline, cut and preserved in 10% buffered formalin immediately after slaughtering for histopathological evaluation.

**Serum analysis:** Liver activity parameters (AST, ALT) and kidney activity parameters (creatinine and blood urea nitrogen) were measured using commercially available diagnostic kits.

**Histometric analysis:** Following fixation, tissues were cut into thin slices (1cm³) with the help of a clean sharp knife. Tissues were processed by paraffin technique, sectioned at 5-7 µm and subjected to Hematoxylin and Eosin (H&E) staining procedure (Bancroft et al., 2013). Slides were examined under microscope at 100X to measure the thickness (µm) of endometrium and myometrium using automated image analysis system, image J®. Vacuolar and degenerative changes in tubules of kidneys and radial arrangement of hepatocytes around central vein and necrosis of hepatocytes were also observed.

**Statistical analysis:** Descriptive statistics of each parameter under study was calculated with the help of computer software Microsoft Excel®. The means of parameters were compared with one-way analysis of variance (ANOVA), Group means were compared with the help of Least Significance Difference (LSD) test. The level of significance was kept at 5%.
293-298 (P<0.01) in combined treatment. Histology of normal liver demonstrated well organized radiating pattern of sinusoidal cords and hepatocytes around central vein (Fig. 2A). Photomicrograph of liver, in diabetic control, showed fatty necrosis and vanished radiating pattern of hepatocytes along with hemorrhages and congested central vein (Fig. 2B). Liver with CM and BS oil, separate or combined, treatments presented hepatoprotective effect with less fat necrosis, minor hemorrhages and nearly organized radiating pattern of hepatocytes around central vein (Fig. 2CDE).

Weekly measurement of blood glucose level showed a significant (P<0.05) decrease in all treated groups throughout trial but this reduction was calculated more significant (P<0.01) in third week of experiment in combined treatment group (Fig. 1). Microscopic findings of normal renal tissue revealed the characteristic structure of renal corpuscles along with renal tubules (Fig. 3A). Sections of diabetic control group demonstrated the renal and tubular damage, shrinkage of glomerulus and urinary space (Fig. 3B). The CM and BS oil, separate or combined, showed protective effect on the histology of kidneys (Fig. 3CDE) with least diabetic tubular destruction and nearly normal renal corpuscles with urinary space.

Data on the histometry of uterus (Table 2, Fig. 4) revealed that diabetes had not significantly (P>0.05) altered thickness of endometrium and myometrium. Similarly, treated groups did not show any alteration in histology of uterus.

**DISCUSSION**

Diabetes causes systemic and metabolic disturbances which eventually result in hyperglycemia. It alters normal metabolism of lipids, proteins and carbohydrates in body and generates free radicals which impair antioxidant system of body (Alimohammadi et al., 2013). Natural products having antidiabetic activity are preferred over synthetic drugs due to economical and safe usage (Baragob, 2015). The present study was conducted to evaluate antidiabetic activity of CM and BS oil either separately or in combination in induced diabetic rats.

![Fig. 1: Weekly fasting blood glucose level of rats of different groups.](image1)

![Fig. 2: Micrograph of liver section: normal (A) demonstrated the normal histological architecture of radiating pattern of hepatocytes (H) and sinusoidal cords (S) around central vein (CV), control diabetic (B) presented the distorted cellular and radiating pattern of hepatocytes (H) and odematous hepatic vein (HV) with hepatic artery (HA) and bile duct, CM (C) and BS (D) treated groups showed improvement in diabetic induced distorted pattern of hepatocytes (H) and sinusoidal cords (S), group with combined therapy (E) drifted the diabetic induced pathological changes towards normal hepatic architecture. H&E: 100X.](image2)
A study,

aphrodisiac properties were observed in rats which are immune booster and prevent cellular injury by

thymoquinone with unsaturated fatty acids respectively. The presence of insulin, vitamin C and zinc

hyperglycemic activities of CM and BS oil may be due to the presence of thymoquinone and high

liver damage due to presence of thymoquinone and high levels of antioxidants. 

Ghoreishi and Zari (2011). The combination of CM and BS oil showed more significant (P<0.05) reduction in liver

enzymes in DM. Elevated levels of liver enzymes are characteristic of DM and can serve as biomarkers of the detrimental impact of DM on liver structure. From where AST and ALT biomarkers of the detrimental impact of DM on liver structure from where AST and ALT respectively.

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The findings of Hassan and Emam (2012). A significant (P<0.05) minimization was seen in fasting blood glucose by CM and BS oil therapy. However, more significant (P<0.05) reduction was seen in fasting blood glucose by combine therapy of these agents. Possible hypoglycemic action of CM and BS oil may be due to the presence of coated insulin like protein in CM, endure stomach pH and increase its absorption. Logman and Eman (2014), Khan et al. (2013). The findings obtained by Alsaif, 2008; Alsaif et al. (2016). DM associated anemia could be attributed to upsurge of non-enzymatic glycosylation of erythrocytes and its indices (Hb, MCV, MCH). Increased lipid peroxidation in hyperglycemia is main culprit of lysis of erythrocyte cell membrane (Oyedemi et al., 2011). In current study, anemic effects of DM on erythrocyte and its indices correspond with Erukainure et al. (2013) findings. Anti-hyperglycemic effect of both CM and BS oil separately significantly (P<0.05) drift the diabetic altered erythrocytes and its indices towards normal. But in combination their significant (P<0.05) anti-diabetic effect was following same pattern as that of separate CM therapy. High levels of antioxidant, magnesium, zinc and phytochemical compounds, down regulate the non-enzymatic glycosylation of erythrocytes and lipid peroxidation by decreasing glucose exposure to the body organs, can be responsible for this protective effect of CM and BS oil respectively.

Leukocytes are the inborn part of the immune system. The diabetes induced synthetic drugs hamper the immune system of animals by deranging the normal physiological leukocytosis (Erukainure et al., 2013). The findings obtained by this study are in consistent with the hematological findings of Edet et al. (2013). Treatment of diabetes with CM and BS oil significantly (P<0.05) improved these diabetogenic leukocyte values separately as well as in combination. The synergistic effect of these agents in DM may be due to some constituents that protect and stimulate leukocytosis in diabetic rats, these constituents and their exact mechanism are yet to be known.

### Table 1: Effect of camel milk and black seed oil on diabetic altered hematological and serological parameters in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBCs (10^12/l)</th>
<th>PLT (10^9/l)</th>
<th>WBC (10^9/l)</th>
<th>MCV (fl)</th>
<th>Hb (g/dl)</th>
<th>Lymphocytosis (%)</th>
<th>MCH (pg)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>8.94±0.12</td>
<td>293.67±72.74</td>
<td>5.63±1.05</td>
<td>3.15±0.42</td>
<td>16.93±0.35</td>
<td>14.72±0.37</td>
<td>74.74±4.23</td>
<td>44.80±1.42</td>
<td>28.50±2.34</td>
<td>11.42±0.37</td>
<td>20.12±0.37</td>
</tr>
<tr>
<td>B (Diabetic control)</td>
<td>0.12±0.01</td>
<td>305.28±66.27</td>
<td>5.62±1.05</td>
<td>3.15±0.42</td>
<td>16.93±0.35</td>
<td>14.72±0.37</td>
<td>74.74±4.23</td>
<td>44.80±1.42</td>
<td>28.50±2.34</td>
<td>11.42±0.37</td>
<td>20.12±0.37</td>
</tr>
<tr>
<td>C (Camel milk treated)</td>
<td>4.81±0.01</td>
<td>307.44±47.09</td>
<td>5.62±1.05</td>
<td>3.15±0.42</td>
<td>16.93±0.35</td>
<td>14.72±0.37</td>
<td>74.74±4.23</td>
<td>44.80±1.42</td>
<td>28.50±2.34</td>
<td>11.42±0.37</td>
<td>20.12±0.37</td>
</tr>
<tr>
<td>D (Black seed oil treated)</td>
<td>0.36±0.01</td>
<td>314.73±52.25</td>
<td>5.62±1.05</td>
<td>3.15±0.42</td>
<td>16.93±0.35</td>
<td>14.72±0.37</td>
<td>74.74±4.23</td>
<td>44.80±1.42</td>
<td>28.50±2.34</td>
<td>11.42±0.37</td>
<td>20.12±0.37</td>
</tr>
<tr>
<td>E (Camel milk + black seed oil treated)</td>
<td>0.36±0.01</td>
<td>325.42±52.74</td>
<td>5.62±1.05</td>
<td>3.15±0.42</td>
<td>16.93±0.35</td>
<td>14.72±0.37</td>
<td>74.74±4.23</td>
<td>44.80±1.42</td>
<td>28.50±2.34</td>
<td>11.42±0.37</td>
<td>20.12±0.37</td>
</tr>
</tbody>
</table>


### Table 2: Effect of diabetes, camel milk and black seed oil on uterine histomery in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uterine Parameters (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Normal)</td>
<td>Endometrium: thickness (μm)</td>
</tr>
<tr>
<td></td>
<td>305.28±66.27</td>
</tr>
<tr>
<td>B (Diabetic control)</td>
<td>Myometrium: thickness (μm)</td>
</tr>
<tr>
<td></td>
<td>307.44±47.09</td>
</tr>
<tr>
<td>C (Camel milk treated)</td>
<td>Endometrium: thickness (μm)</td>
</tr>
<tr>
<td></td>
<td>314.73±52.25</td>
</tr>
<tr>
<td>D (Black seed oil treated)</td>
<td>Myometrium: thickness (μm)</td>
</tr>
<tr>
<td></td>
<td>325.42±52.74</td>
</tr>
<tr>
<td>E (Camel milk + black seed oil treated)</td>
<td>Endometrium: thickness (μm)</td>
</tr>
<tr>
<td></td>
<td>298.67±72.74</td>
</tr>
</tbody>
</table>

Means in columns are statistically similar (P<0.05).
Fig. 3: Photomicrograph of kidney section: normal (A) demonstrated the normal histological architecture of glomerulus (G), urinary space (US), proximal convoluted (PT) and distal convoluted tubules (DT); control diabetic (B) showed distorted glomerular (G) structure along with severe tubular degeneration (D) and reduced urinary space (US); CM (C) and BS (D) treated groups showed improvement in diabetic induced tubular (D) glomerular (G) degenerations; group with combined therapy (E) drifted the diabetic induced pathological changes in histology of kidney towards normal renal architecture. (H&E): 100X.

Fig. 4: Photomicrograph of uterus section: normal (A); control diabetic (B); camel milk treated (C); black seed treated (D); group with combined therapy (E) showed thickness of endometrium (END) and myometrium (MYO). (H&E): 100X.
Platelets are the slices of cell that initiate and take part in blood clotting and repair process. The possible implication of significant (P<0.05) decreased platelet count in DM is that free radicals generated by alloxan destroy the blood clotting mechanism which consequents in internal bleeding (Oyedemi et al., 2010). A significant (P<0.05) recovery was seen in platelet count by CM and BS therapy. However, CM in combination with BS showed no synergistic protective effect.

Alloxan® increased the oxidative stress due to which liver and kidney become more susceptible to oxidative injury and altered normal histology. These histological degenerations in hepatic and renal tissues, found in this experiment, were in accordance to the Ragavan and Krishnakumari (2006) and Aboonabi et al. (2014). CM and BS oil potentially recovered the normal cellular architecture of these tissues, while more curative effect was observed in combined treatment. Presence of antioxidant vitamins (A, B, C and E), insulin and zinc in CM (Mullaicharam, 2014) and thymoquinone in BS oil (Mollazadeh and Hosseinzadeh, 2014) can be held responsible for this recovery of diabeticogenic alterations in liver and kidney structure towards normal. The above mentioned constituents, integral part of more than 300 enzymes of the body, have potential to decrease oxidative stress by activating antioxidant system and stimulating the enzymes of the body, have potential to decrease oxidative stress in strongly affected diabeticogenic tissues.

Favaro et al. (2010) described that DM showed its effect on uterus in tissue sensitive matter. Only chronic form of DM (>60 days) showed detrimental impact on uterine histology in rats. Induced hyperglycemia for short period of time (8 week) did not reveal any significant alteration in uterine histometry. Data explored in the present study was in accordance to the Favaro et al. (2010). This may be due to non-significant diabeticogenic alternation in the uterine vasculature and nerve innervation.

Conclusions: Results of the present study showed that camel milk and black seed oil have synergistic effect with the potential to recover diabetes related hematological and serological complications and to constraint the diabetic encounter on liver and kidneys’ histology. These results may have implications in the clinical management of diabetes mellitus in human.

Authors contribution: MZ Ali and AS Qureshi designed the project, supervised the work and contributed in the preparation of manuscript. M Usman, R Kausar and MK Ateeq performed laboratory sampling, statistical analysis and preliminary write up.

REFERENCES

