Efficiency of Camel Milk and Honey Bee in Alleviation of Diabetes in Rats

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Abstract: Diabetes elicits an increase in the oxidative stress-mediated endothelial dysfunction. Fifty male rats were divided into two main groups, first group served as control group was considered as normal non-diabetic (n=10). Second group (n=40): rats were subcutaneously injected with alloxan (150 mg/kg. body weight) for induction of diabetes. Then the diabetic rats were divided into four equal subgroups as follows comprising of ten animals each: first was diabetic rats , and the second group was received camel milk -treated diabetic group that was treated with camel milk at a dose 40 ml/rat daily for four weeks. The third group was received camel milk as previous combined with honey bee - treated diabetic group. The fourth is honey bee dose of 10ml honey/kg/5ml of distilled water diluted for four weeks. Results show that the camel milk either alone or combined with honey bee significantly reduced the hyperglycemia from 217.69±0.70 nmol/µl (Diabetic untreated rats) to 126.8±0.68 nmol/µl and 115.90±0.60 respectively. While, the previous treatment significantly increased insulin hormone, insulin growth factor 1(IGF-1) and interferon gamma-γ as well as, lysozyme, glutathione peroxidase (GPx) and nitric oxide (NO) as compared to the untreated rats. On the contrary, the level of IFN-γ in serum was increased in alloxan administered animals, which was decreased significantly in rats had camel alone or with honey bee. In conclusion, the observations from this study show that camel milk and or honey bee has hypoglycemic effect on experimental diabetic rats. [Nature and Science 2010;8(10):333-341]. (ISSN: 1545-0740).

Keywords: Camel milk; honey bee, alloxan induced diabetes; hyperglycemia

1. Introduction:
Diabetes mellitus remains a global major health problem in the World over with the tropics inclusive. Diabetes is a metabolic disorder that is known to produce various dysfunctions in the body including the central nervous system (CNS). Some of the diabetes related CNS disturbances include hyperphagia, polydypsia and activation of the hypothalamo–pituitary–adrenal axis (Biessels et al., 1994). The sustained hyperglycemia leads to a further impairment of insulin production by β-cells, so called glucose toxicity (Del Prato and Marchetti, 2004). In addition, the elevated serum triacylglycerol and its accumulation in pancreatic islets during the development of diabetes have been associated with impaired β-cells secretory responses (Ishikawa et al. 2008).

Camel milk has an adjuvant effect to insulin therapy in control of diabetes (Agarwal et al., 2005). Raw camel milk against type 1 diabetes has shown encouraging results and average daily insulin requirements showed a decrease of about 30-40 percent in 92% of patients. Camel milk consumption may also be helpful in reducing the nutritional deficiencies and morbidities in adult community (Singh et al., 2009). A series of metabolic and autoimmune diseases are successfully being treated with camel’s milk (Al-Hashem, 2009).

Agarwal et al. 2009 reported that, a significant effect of camel milk on microalbuminuria when given as adjunctive therapy in type 1 diabetic patients but the reason behind this is still unknown. Further studies on camel milk are necessary to identify the components which are responsible for lowering microalbuminuria levels. It was also concluded that camel milk shows its significant hypoglycemic effect when given along with conventional treatment. The action is presumed to be due to the presence of insulin/insulin like protein. Its therapeutic efficacy may be also due to the lack of coagulum formation in acidic media. Increased oxidative stress and reduced nitric oxide (NO) bioavailability are key features of diabetes mellitus that may result in vascular dysfunctions (Capellini et al.,2009).

Natural honey is widely used all over the world as a complementary and alternative medicine in various disorders including gastrointestinal lesions. Although honey is a high carbohydrate food, its glycemic index varies within a wide range from 32% to 85%, depending on the botanical source. It contains small amounts of proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols (Bogdanov et al., 2008). Increased oxidative stress and reduced nitric oxide (NO) bioavailability are key features of diabetes mellitus that may result in vascular dysfunctions (Capellini et al., 2009). Several alternate therapies include honey as
an important component in the management of diabetes but the mechanism for its hypoglycemic effect has not been clearly understood. Elevation of plasma insulin levels and lowering of blood glucose levels have been observed in patients with diabetes after administration of honey (Al-Waili, 2004). Honey contains a high concentration of fructose, a monosaccharide, capable of raising blood sugar level after oral ingestion. It is thus a paradox that nutritional experts have advocated its use as a nutrition supplement in patients with diabetes mellitus. It has also been used, over the years, as a sweetener by those who wish to avoid the use of sugar. The effective use of sugar in diabetes may be due to its other constituents, especially the various antioxidants that are abundant in honey (Fasanmade and Alabi 2008). The therapeutic properties of honey, once considered a form of folk or preventive medicine, are acquiring importance for the treatment of acute and chronic free radical-mediated diseases (atherosclerosis, diabetes and cancer) (Beretta et al., 2007).

In this connection we have heard of many stories which describe the usefulness and acceptability of camel milk and its use in the treatment of diabetes mellitus. One of the camel milk proteins has been reported to have similar characteristics to insulin (Agrawal et al., 2004). Camel milk does not form coagulum in acidic environment. The lack of coagulum formation allows the camel milk to pass rapidly through the stomach together with the specific insulin like protein/insulin and remains available for absorption in intestine. Radioimmunoassay of Insulin in Camel milk has revealed high concentration i.e. 52 units/liter (Singh, 2001). Prevention and early treatment is important because diabetes interrupts normal developments in children and carries the threat of severe complication in more active period of life (Agrawal et al., 2004).

Surprisingly, camel milk does seem to contain high levels of insulin or an insulin-like protein which appears to be able to pass through the stomach without being destroyed (Agrawal et al., 2004). The stomach's acidity would normally destroy insulin – this is why developing 'oral insulin' is such a challenge.

Anecdotal reports suggest a very low prevalence of diabetes among subjects that are consuming camel milk (Agrawal et al., 2004). This study was carried out to evaluate the impact of camel milk and / or honey bee on diabetic rats. Furthermore, these treatments on improvement of insulin sensitivity and biochemical complications of alloxanized diabetic albino rats and to suggest their probable hypoglycemic mechanisms.

2. Materials and Methods:

Camel’s milk samples and honey: Milk samples and pure honey bee were obtained from farm in Bilbis area (South to Cairo).

Experimental design:

Fifty Male albino rats weighing (180 - 200 gm.) were acclimatized under laboratory conditions for a week by keeping them on standard rat chow and water ad libitum. Animals were housed in a temperature, humidity and light controlled room (temperature 22 °C, humidity 50%, 12h light, and 12h darkness). After fasting for 24 hrs, animals received a single intraperitoneal injection of freshly prepared alloxan (Sigma chemical) using 2% sodium citrate solution 0.05M (pH = 4.5) as vehicle, at a dose of 150 mg alloxan/kg body weight (Szkudelski et al., 1998). Alloxan (Sigma Chemical Company USA) with a pH of 7.0 was kept at 37°C before injection.

Animal grouping control group (Gp. I) was considered as normal non diabetic rats. Diabetic group (Gp. II) was diabetic untreated rats.

Camel milk -treated diabetic group was diabetic treated with camel milk (Gp. III & IV) were given 40 ml of camel milk for each rat daily for 4 successive weeks by oral cannula to achieve the best possible glycemic control in alloxan- induced diabetic rats (Agarwal et al., 2005). In group IV, 40 ml of camel milk was additionally added to honey bee. Group V, rats were given honey bee daily for 4 successive weeks. Each rat in Gp IV &V honey received a daily dose of 10ml honey/kg/5ml of distilled water (Busserolles et al., 2002) through an oral cannula.

Blood samples were collected from orbital venous plexus of the experimental animals from overnight fasted rats. Serum was separated for different biochemical analysis.

Biochemical investigations:

Serum was collected for analyses of the following parameters: Glucose was determined by fluorometric assay using kit purchased from Bio Vision’s kit USA., insulin hormone was determined by radioimmunoassay (RIA) according to (Marschner et al., 1974) and insulin-like growth factor (IGF-1) for rats were done by ELISA according to the techniques of (Daughaday and Rotwein 1989) Glutathione peroxidase (GPx) activity and nitric oxide were determined by using a colorimetric kit (BioVision, USA.). Lysozyme and rat interferon gamma (IFN-γ) were determined in serum according to (Borgen and Romslo, 1977 and Stachelin et al. 1981 and Kelder et al. 1986 respectively).

Statistical Analysis:
Values were recorded as Mean ± standard error of the mean. Statistical difference between the means was determined by ANOVA followed by Duncan post Hoc test. P< 0.05 was accepted as significant level.

3. Results:

As shown in table (1) a decline (P<0.05) in the blood glucose level in milk camel group when compared to diabetic untreated group was 126 ± 0.68 & 217.95 ± 0.70 respectively. While in combined treatment group (camel milk and honey bee) there was a significant decrease (P<0.05) of glucose as compared to diabetic untreated group 115.90 ± 0.60 this decrease (P<0.05) (Table 1).

There was a significant decrease (-70.79%) in the level of insulin in the group was treated with alloxan compared to the control group. The treatment with camel milk and honey bee either separately or combined with each other had ameliorated effects on insulin levels when compared to the diabetic untreated group (Table 1). Similarly, there was a significant decrease of IGF1 in diabetic group compared to the control(46.90%). Since camel milk, honey bee separately and combined treatment increased of IGF1 compared to diabetic untreated rats. The increase (P<0.05) was observed in the combined group (camel milk and honey bee) this increase amounting by 60.25 % (Table 1).

Table (2) shows the levels of lysozyme, GPX and NO there were significant decrease (P<0.05) of these parameters in diabetic untreated group compared to the control group. Camel milk, honey bee either separately or combined increase significantly (P<0.05) of lysozyme compared to diabetic untreated rats by 52.17%, 28.21% and 83.67% respectively (Table 2). As well as GPX increases significantly (P<0.05) after treatment of camel milk and combined treatment (camel milk and honey bee) compared to the untreated diabetic group (650.75 ± 2.32 and 750.50 ± 3.33 respectively). There was a significant increase (P<0.05) of NO in diabetic untreated rats compared to the control amounting by 54.56%. The level of NO in the groups treated with camel milk, honey bee separately and combined was increased significantly by 52.78%, 25.21% and 74.57% respectively compared to untreated group. The decrease (P<0.05) was observed after combined treatment (Table 2).

Likewise, Table (2) shows a significant increase (P<0.05 & 70.5%) of INF-γ in alloxanized diabetic group compared to that of non-diabetic ones. The treatment with camel milk, honey bee individually and combined led to decrease significantly of INF-γ. The significant decrease (P<0.05) was observed in the group which treated with camel milk plus honey bee (-37.26%) when compared to untreated group (Table2).

4. Discussion:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia may cause high production of free radicals and consequently increase of the oxidative stress. Pancreatic β cell destruction with alloxan has been successfully used in the induction of type 1-like diabetes mellitus in laboratory animals.

The present data demonstrated that alloxan diabetic rats showed marked carbohydrate metabolic disturbances including severe hyperglycemia, hypoinsulinemia. Hyperglycemia can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on the cells of the pancreas (Bolaffi et al., 1986). Also from the biochemical point of view, it was evident that increased glucose in diabetes mellitus may be derived either from glycogenolysis or from gluconeogenesis or both (Shibib et al., 1993, Rawi et al., 1996). This study demonstrated that the oral administration of camel milk and/ or honey bee caused marked amelioration of serum glucose concentration of alloxan diabetic rats. Amino acid sequence of some of the camel milk protein is rich in half cystine, which has superficial similarity with insulin family of peptides (Beg et al., 1986).

Administration of camel milk combined with honey bee caused marked amelioration of serum glucose concentration of alloxan diabetic rats, besides elevating insulin concentration and IGF-1.

IGF-1 that promotes glucose as energy substrate by stimulating peripheral uptake and glucose oxidation instead of fat by suppressing lipolysis (Jacob et al., 1989), may further contribute to the reduction of and the carbon dioxide production volume, because glucose oxidation is less oxygen demanding than is fat oxidation (Jacob et al. 1989 and Froesch et al. 1993). IGF-1 has been reported to confer cytoprotection against ischemia and reperfusion injury and thus to accelerate recovery of post ischemic cardiac function (Otani et al., 2000). The insulin like effect of IGF-1 to stimulate glucose uptake and oxidation may be potentially beneficial in postoperative patients. Normalization of blood glucose levels with intensive insulin therapy reduced mortality and morbidity among critically ill patients in the surgical intensive care unit, regardless of whether they had a history of diabetes (Van den Bergh, 2001). Although the mechanisms remain a matter of speculation. IGF-1 has been shown to be 6% as potent a hypoglycemic agent as insulin (Guler et al., 1989).

Activities of IFN-γ was reduced by camel milk combined with honey bee. IFN-γ is a well-described macrophage activating factor and generally is considered to be a pro-inflammatory cytokine.
(Gazzinelli et al., 1992). It enhances certain macrophage functions, such as microbicidal and tumoricidal activity, via increased IFN-γ is a well-described macrophage activating factor and generally is considered to be a pro-inflammatory production of reactive oxygen intermediates and reactive nitrogen intermediates (Flesch et al., 1994 and Ohmori and Hamilton 1994). Moreover, IFN-γ is a Th1-type, pro-inflammatory cytokine that is actively involved in almost all phases of immune and inflammatory responses, including macrophage activation, antibacterial immunity, antigen presentation, activation of the innate immune system, lymphocyte-endothelium interactions, Th1/Th2 balance, and cellular proliferation and apoptosis. IFN gamma is primarily secreted by T cells (Gattoni, et al., 2006). Several reports have shown an increase in the activities of IFN-γ in the insulitis process may be involved in the pathogenesis of type -1 diabetes (Cardozo, et al., 2003 and Foulis et al., 2005). It is apparent, however, that addition of honey to the camel milk results in further increase in the level of IFN-γ, lysozyme and NO irrespective of treatment. As well as, the antioxidant properties of lysozymes are partly mediated by a reduction of ROS levels and of stress response genes (Napoli et al., 2003 and Peng et al., 2004). The distinct qualities of honey as a useful agent in medical practice may be due to its unique components (Iftikhar et al., 2009).

Table 1: Effect of camel and honey bee separately or combined on various biological parameters in diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Diabetic- group untreated</th>
<th>Diabetic - treated group</th>
<th>Camel milk only</th>
<th>Honey only</th>
<th>Camel milk + honey bee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (nmol/µl)</td>
<td>99.69±0.74e</td>
<td>217.69±0.70a</td>
<td>126.8±0.68e</td>
<td>150.80±1.27b</td>
<td>115.90±0.60d</td>
<td></td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>118.37</td>
<td>27.19</td>
<td>51.27</td>
<td></td>
<td>16.26</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td></td>
<td>-41.75</td>
<td>-30.73</td>
<td></td>
<td>-46.76</td>
<td></td>
</tr>
<tr>
<td>Insulin (µ IU /ml)</td>
<td>32.08 ± 0.66a</td>
<td>9.37 ± 0.15c</td>
<td>25.35 ± 0.22c</td>
<td>16.50 ± 0.23d</td>
<td>29.48 ± 0.35b</td>
<td></td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>-70.79</td>
<td>-20.98</td>
<td>-48.57</td>
<td></td>
<td>-8.10</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td></td>
<td>170.54</td>
<td>76.09</td>
<td></td>
<td>214.62</td>
<td></td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>402.41±1.11a</td>
<td>213.68±1.10c</td>
<td>295.26±1.02c</td>
<td>232.83±0.79d</td>
<td>342.42±1.36b</td>
<td></td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>-46.90</td>
<td>-26.63</td>
<td>-42.14</td>
<td></td>
<td>-14.91</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td></td>
<td>38.18</td>
<td>8.96</td>
<td></td>
<td>60.25</td>
<td></td>
</tr>
</tbody>
</table>

Values represent means ± S.E. P< 0.05
Values with same superscript in the raw are not statistically different.
Table 2: Effect of camel and honey bee separately or combined on various biological parameters in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>Diabetic- group untreated</th>
<th>Diabetic - treated group Camel milk only</th>
<th>Honey only</th>
<th>Camel milk +honey bee</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-γ (pg/ml)</td>
<td>385.46±0.96e</td>
<td>657.20±1.04a</td>
<td>468.2±1.44e</td>
<td>503.20±0.61b</td>
<td>412.3±1.13d</td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>70.50</td>
<td>21.47</td>
<td>30.55</td>
<td>6.96</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td>-28.76</td>
<td>-23.43</td>
<td>-37.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme (mg/l)</td>
<td>49.0±0.23a</td>
<td>19.78±0.28e</td>
<td>30.10±0.49d</td>
<td>25.36±0.32c</td>
<td>36.33±1.03b</td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>-59.63</td>
<td>-38.57</td>
<td>-48.24</td>
<td>25.86</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td>52.17</td>
<td>28.21</td>
<td>83.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPx (mU/ml)</td>
<td>798.30±1.44a</td>
<td>525.11±3.56c</td>
<td>650.75±2.32c</td>
<td>590.89±1.82d</td>
<td>750.50±3.33b</td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>-34.22</td>
<td>-18.48</td>
<td>-25.98</td>
<td>-5.99</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td>23.93</td>
<td>12.53</td>
<td>42.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO (nmol/µl)</td>
<td>10.30 ± 0.29d</td>
<td>4.68 ± 0.14e</td>
<td>7.15 ± 0.22c</td>
<td>5.86 ± 0.26d</td>
<td>8.17 ± 0.22b</td>
</tr>
<tr>
<td>Change% (Control)</td>
<td>-54.56</td>
<td>-30.58</td>
<td>-43.11</td>
<td>-20.68</td>
<td></td>
</tr>
<tr>
<td>Change% (Untreated)</td>
<td>52.78</td>
<td>25.21</td>
<td>74.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent means ±S.E. P< 0.05
Values with same superscript in the raw are not statistically different.

Alloxan induced significant decreased of lysozyme (LZ) activity this result was is good agreement with other investigators Lechowski and Lenarcik 1991 who reported that serum lysozyme Activity reduction test in dogs with diabetes mellitus. The in vivo overexpression of the native defense protein LZ can protect against acute oxidative stress, as well as confer resistance to chronic oxidative stress against milder oxidants (Liu et al., 2006). GPx activity significantly decreased in diabetic group administrated alloxan.

This significant increase in GPx activity in diabetic rats treated with camel milk and /or honey bee may have been a response to the increased peroxidative stress from the high SOD activity which converts O2 - into H2O2. This may demonstrate that camel milk and / or honey bee in diabetic rats promoted GPx activity, which acted to decrease peroxidative stress. This data is in agreement with (Ndahimana et al., 1996). However, hyperglycemia increases ROS production and consequently oxidative stress (Rolo & Palmeira, 2006), and this situation promotes increased inflammatory factors (Guo et al., 2007). Possibly the destruction of pancreatic beta cells by alloxan-induced ROS generation (Szkudelski, 2001 and Lenzen, 2008). The potentially therapeutic effect of camel milk and /or

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honey bee on these cells, and consequently on hyperglycemia status.

During diabetes there is increased production of oxygen free radicals (OFRs) through glucose autoxidation and protein glycation (Wolff and Dean 1987 and Hunt et al., 1990). The oxidative degradation of these oxidants could participate in the formation of lipid peroxidation products. The oxidative degradation of fructosamines may contribute to the oxidative stress found in hyperglycemia associated with diabetes mellitus (Agarwal et al., 2004). There could be other sources of generation of free radicals such as immune mechanisms which are implicated in the pathogenesis of diabetes mellitus (Hussain et al., 1996). Moreover, these previous authors have shown that serum levels of macrophage-derived cytokines are increased well before the onset of diabetes mellitus. The activation of macrophages could lead to the production of OFRs. Under diabetic conditions increased production of several reduced sugars through glycolysis or polyol pathway results in oxidative stress. GPx plays a primary role in minimizing oxidative damage through the increase in the GPx activity in the liver and pancreas suggests its induction by both higher organic and inorganic peroxides (Ho et al., 1997). Also, an increase in GPx mRNA at higher concentrations of H2O2 (Shull et al., 1991).

A significant decrease of NO in diabetic untreated group, It is possible that reduced NO activity contributes to the increased cardiovascular morbidity observed in diabetes (Tsao et al.,1994).

Nitric oxide, an important mediator in diabetic wound healing and collagen synthesis, was measured in wound fluid. Wound-derived fibroblasts were tested for ex vivo synthesis of nitric oxide and collagen (Schaffer et al., 2007). Vascular NO activity is reduced in diabetes, leading to impaired endothelium-dependent vasodilation (Giugliano and Ceriello 1996) and elevated platelet aggregation. (Tsao et al., 1994 and Shukla et al., 1992).

Honey also caused a reduction in hyperglycemia induced by long-term ingestion of camel milk, Honey could not reduce blood glucose in controlled rats that received alloxan treatment as combined treatment (camel milk plus honey bee).

Hyperglycemia develops after alloxan administration and stimulates the production of advanced glycosylated end-products, enhances the activates protein kinase C (Baynes, 1991). These conditions might lead to increased generation of ROS, such as superoxide anion (O2−) (Nishikawa et al., 2000), which rapidly reacts with NO leading to the formation of ONOO−, which is highly oxidant and capable of damaging several biological molecules (Chiueh, 1999). Honey is composed of minerals like magnesium, potassium, calcium, sodium chloride, sulphur, copper, iodine, zinc, iron and phosphate. It also contains vitamins B1, B2, C, B6, B5 and B3, all of which changed according to the qualities of the nectar and pollen. The anti-oxidant effects of honey (Gheldof et al 2002) would thus make it a useful adjunct in the management of diabetes mellitus.

The mechanism for the hypoglycemic effect of honey is, however, not well understood. Honey is a mixture of sugars – fructose (about 38.5%) and glucose (about 31.0%), maltose, sucrose and other complex carbohydrates. One would thus expect that consumption of honey would raise the blood sugar and that in fact the glycemic index of honey should approach that of glucose. The finding in several studies revealed that honey causes a reduction in blood glucose levels in both normal and diabetic patients and this is an indication that honey has a mechanism, probably insulin sensitization effect (Al-Waili, 2004). The protective effect of Camel’s milk against diabetic's oxidative stress in the rats is due to its antioxidant properties and possible chelating effects on free radicals. Camel’s milk was found to contain high concentrations of vitamins A, B2, C and E and is very rich in magnesium and other trace elements (Yousef, et al., 2004 and El-Said et al., 2010). These vitamins act as antioxidants and have been useful in preventing toxicant- induced tissue injury. Surprisingly, camel milk does seem to contain high levels of insulin or an insulin-like protein which appears to be able to pass through the stomach without being destroyed (Chaillous et al., 2000). The stomach's acidity would normally destroy insulin, this is why developing 'oral insulin' is such a challenge to presence of insulin/insulin like protein.

5. Conclusion:
In conclusion, the current study indicates that the study shows a significant hypoglycemic effect of camel milk and /or honey bee.

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