THE EFFECT OF *BOSWELLIA CARTERII* ON MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IMMUNE MARKER IN DIABETIC PATIENTS

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ABSTRACT

Diabetes mellitus (DM) commonly called to as diabetes is a set of metabolic disorders in which there are high blood sugar levels over a prolonged time. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin. *Boswellia Carterii* is one of the most powerful natural inflammatory agents, antioxidants making it a natural tonic for the body's immune system. Aim of the study is evaluate and estimate the effect of natural product *Boswellia Carterii* on Monocyte chemoattractant protein 1(MCP1) immune marker, HbA1c and FBS in diabetes patients after and before one month of treatment.

In this study the total of 25 patients suffering from diabetic mellitus type 2 was submitted to this study with age range of 40-67 years in addition to another 25 cases from healthy persons used as control. The patient took 600 mg from *Boswellia carterii* once daily for one month as capsule. Blood samples of Diabetic patients take to measuring the FBS by reflotron, HbA1c by kit and MCP-1 by ELIZA.

HbA1c & FBG decreased significantly (**P<0.01) after treatment with *Boswellia Carterii* compared to normal cases, while no change on MCP-1 level was observed after one month of treatment. In addition, there were significant correlation between MCP-1 and FBG * (P<0.05) and between FBG and HbA1c ** (P<0.01).

*Boswellia Carterii* good therapy to reduce glucose and, HbA1c level in diabetes but not MCP-1. This lead to give idea about the dose which gave to the DM patients in this study (600mg/one dose daily) was low to effect on cytokines.

Keywords: diabetic mellitus, Boswellia Carterii, Monocyte chemoattractant protein 1(MCP1)

INTRODUCTION

Diabetes mellitus (DM): commonly called to as diabetes is a set of disorders in which there are high blood sugar levels over a prolonged time \(^1\) sign and Symptoms of high blood sugar include increased hunger, frequent urination and increased thirst.. If left untreated, diabetes can cause many complications as showed in Figure (1) include Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death while chronic complications include chronic kidney disease, cardiovascular disease, damage to the eyes, stroke, and foot ulcers\(^2,3\).

Diabetes is as a result of either the pancreas not producing an adequate amount of insulin, or the cells of the body not responding appropriately to the formed insulin\(^4\). There are three main types of diabetes mellitus \(^2\). The world prevalence percent of diabetes may reach to 55\% between 2013 and 2035\(^5\).

Obesity is an important risk factor for type 2 diabetes, and with global obesity rates rising, the trouble with type 2 diabetes may beget worse \(^6\). These previous findings indicate that a large percentage of the Iraqi population is at risk of increasing type 2 diabetes mellitus in the next years. The high prevalencerates offamily history of obesity, diabetes and inactivelifestyle raise the need for a lifestyle focus on persons at risk of DM type 2 \(^7,8\).

Monocyte chemoattractant protein 1(MCP1) is also referred to CCL2 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, dendritic cells and memory T cells to the sites of inflammation formed by injury of tissue or by infection\(^9,10\). MCP1 associated with many diseases characterized by monocytic infiltrates cells, for example psoriasis, atherosclerosis and rheumatoid arthritis\(^11\). Experimental autoimmune encephalomyelitis (EAE), and traumatic brain injury\(^12\). In addition to that, the CCL2 plays an main role in the vascular complications of DM type 2 \(^13\).

*Boswellia Carterii* are large trees that are widely grown in many regions around the world including the Asian regions and the northern part of the African continent but the main home is traced back to Yemen \(^14\). It is consider one of the most powerful natural antioxidants making it a natural tonic for the body's immune system, anti-inflammatory agents, regulates the estrogen hormone \(^15\). Finally, it helps to regulate the blood sugar and prevents the incidence of high and helps reduce in a record time which makes it very useful for diabetics patients of different types especially the second type \(^16\).

Aim of the study is evaluate and estimate the effect of natural product called *Boswellia Carterii* on Monocyte chemoattractant protein 1(MCP1) immune marker, HbA1c and FBS in diabetes patients after and before one month of treatment.

MATERIALS AND METHODS

Patients and Samples

Total of 25 patients suffering from diabetic mellitus type 2 was submitted to this study with age range 40-67 years in addition to another 25 cases from healthy persons used as control. The patient take 600
mg from *Boswellia Carterii* once daily for one month as capsule. After one month the 6 ml blood samples were collected from follow up patients 3ml was putted in EDTA tube and the rest 3 ml in the plane tube.

**Glucose measurement (FBS) / Roche Swiss multinational company**

The blood samples were collected from diabetic patient after and before treatment with *Boswellia carterii* capsules. Serum was separated by centrifuge and stored at -4 C till use after one month the glucose level was measured using reflotron tool as follow:

Blood sample was collected and then separated by centrifuge for 5 minute at 4000 rpm to obtain serum. The glucose strip was opened and pipetted the serum (0.56 -33.3 mmol/L) into red point site in the strip by special pipette for this device, then the strip was inserted into reflotron to read the result.

**HbA1c Measurement**

Also serum samples after and before treatment with *Boswellia carterii* capsules used to measure the A1c by on-call A1c kit as follow:

Five microliter of blood sample was collected and put it into R1 tube. R1 tube was incubated for 3 min. reverse it 5-10 times both at the beginning and end of the incubation, then added25 microliter of the mixture to test cartridge. after 15 second later, added 25 microliter from R2. Wait for 1 minute at room temperature. Finally, inserted the test cartridge into the analyzer port to read the result.

**Measurement of MCP1**

The MCP1 was measured duplicate in all patients and control by take the serum and submitted it ELISA Technique procedure as follow:-

Labeled removable 8-well strips as appropriate for your experiment. Added 100 μl of each standard and sample into wells. Covered wells were Covered and incubated for 2.5 h. at room temperature with shaking. The solution Discarded and wash 4 times with 1X Wash Solution. Wash Buffer (300 μl) was added. Removed any remainingWash Buffer by aspirating or decanting. Upturned the plate and blot it next to clean paper towels. 100 μl of 1X prepared biotinylated antibody was added to each well. Incubated for 1 hour at room temperature with shaking. Discarded the solution and re wash. Added 100 μl of prepared Streptavidin solution to each well and Incubated for 45 minutes at room temperature with shaking. Again discarded the solution and repeated the wash. 100 μl of TMB One-Step Substrate Reagent (Item H) was added to each well then Incubated for 30 minutes at room temperature in the dark with shaking. Finally, added 50 μl of Stop Solution (Item I) to each well and read at 450 nm directly.

**RESULTS**
FBG was evaluated in DM patients before treatment (155.32± 3.86) and after treatment (139.28 ± 3.23) as Mean ± SE compared with control patients(84.68 ± 0.9). Significant decrease of FBG observed after treatment with Boswellia Carterii** (P<0.01) as shown in Tab(1).

According to Tab (2), HbA1c in patients was reduced significantly after treatment with Boswellia Carterii(7.19±0.09) versus to DM patients before treatment and control group(8.00 ± 0.19,4.76-+0.13) respectively.

Tab(3) and Fig(1) showed that Mean ± SE of MCP-1 immune marker not affected by treatment with Boswellia carterii in DM patients (115.06± 41.72) compared to DM patients before treatment (114.73± 25.84), while there is significant difference between these Two patient groups and control group(* (P<0.05).

Table 1: FBG estimation before and after treatment with Boswellia Carterii in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Mean ± SE of FBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>84.68 ± 0.91 c</td>
</tr>
<tr>
<td>DM before treatment</td>
<td>25</td>
<td>155.32± 3.86 a</td>
</tr>
<tr>
<td>DM after treatment</td>
<td>25</td>
<td>139.28 ± 3.23 b</td>
</tr>
<tr>
<td>LSD</td>
<td>----</td>
<td>8.346 **</td>
</tr>
</tbody>
</table>

** (P<0.01),Means having with the different letters in same column differed significantly

Table 2: HbA1c estimation before and after treatment with Boswellia Carterii in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean ± SE of HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>4.76 ± 0.13 c</td>
</tr>
<tr>
<td>DM before treatment</td>
<td>25</td>
<td>8.00± 0.19 a</td>
</tr>
<tr>
<td>DM after treatment</td>
<td>25</td>
<td>7.19 ± 0.09 b</td>
</tr>
<tr>
<td>LSD</td>
<td>----</td>
<td>0.416 **</td>
</tr>
</tbody>
</table>

** (P<0.01),Means having with the different letters in same column differed significantly
Table 3: MCP-1 immune marker estimation before and after treatment with *Boswellia Carterii* in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Mean ± SE of MCP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>55.03 ± 2.10b</td>
</tr>
<tr>
<td>DM before treatment</td>
<td>25</td>
<td>114.73 ± 25.84a</td>
</tr>
<tr>
<td>DM after treatment</td>
<td>25</td>
<td>115.06 ± 41.72a</td>
</tr>
<tr>
<td>LSD</td>
<td>----</td>
<td>42.969 *</td>
</tr>
</tbody>
</table>

* (P<0.05), Means having with the different letters in same column differed significantly

Correlation coefficient was evaluated between all parameters in the current study. There is significant correlation between MCP-1 and FBG * (P<0.05), in addition to significant correlation between FBG and HbA1c ** (P<0.01). There is no correlation between the rest parameters as illustrated in Tab (4).
### Table 4: Correlation coefficient between all parameters in this study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient-r</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1 and FBG</td>
<td>0.25</td>
<td>*</td>
</tr>
<tr>
<td>MCP-1 and HbA1c</td>
<td>0.17</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1 and Age</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c and Age</td>
<td>-0.07</td>
<td>NS</td>
</tr>
<tr>
<td>FBG and HbA1c</td>
<td>0.78</td>
<td>**</td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01), NS. Non-Significant.

**DISCUSSION**

Current research showed that the effect of *Boswellia carterii* on diabetic mellitus patients after treated them for one month. In this study many parameters were investigated include FBG, HbA1C and level of MCP1as immune marker. The new data showed that the fasting blood sugar and HbA1C decreased significantly after treatment with *Boswellia carterii* (BC) (P<0.01) in follow up patients (Tab.1) and (Tab.2) respectively. In the Egyptian report, the effect of BC on carbohydrate metabolism in male albino rats by injection the rats with alloxan (to induce diabetic) that led to significant effect include decrease in body weight, liver glycogen and serum insulin with significant increase in blood glucose level (P> 0.01) as versus to control group. After four weeks of treatment, the mean levels of serum glucose for diabetic rat groups treated with the BC were significantly decreased in comparison to the diabetic control group [17].

The decrease blood sugar may be because the effect of *Boswellia carterii* will increase insulin secretion. Hypoglycemic effects of *Boswellia carterii* may be attributed to the main components of this resin, including phenyl propanoids, terpenoids, phenolic compounds and flavonoids which were detected by phytochemical assay by thin layer chromatography [18,19]. Treatment with BCB led to correction of the hypoinsulinaemia which may be due to the regeneration of the β cells of islets of Langerhans [17] and by direct protective effect on β cells through its antioxidant action [20].
Recent study demonstrated that no therapeutic effect of this natural product on the level of MCP-1 cytokine in DM patients after treatment as clearly appeared in Tab (3) and Fig (1). One study reported that the dose of this drug ranged between 200-400 mg/kg was enough to decrease the inflammation \[^{21}\]. This lead to give idea about the dose which gave to the DM patients in this study (600mg/one dose daily) was low to effect on this cytokines. The correlation coefficient was positive between MCP-1 cytokine and FBG but not with HbA1C (Tab.4), this may be because the same reason. Also 25 DM patients included in this study consider low number; therefore we need to increase the number of patients to obtain a good data.

While new data not in line with study reported that Hyperglycemia is the main cause of diabetic angiopathy. High glucose treatment on endothelial cells isolated from diabetic samples resulted an increase of MCP-1 release to 40-70% percent \[^{22}\]. A high glucose level in the cell culture (mesangial cells) activates nuclear factor-Kb (NF-kB) through PKC and reactive oxygen species with stimulates the expression of MCP-1 in human \[^{23,24}\]. Furthermore, high blood glucose was related with production of advanced glycation end products (AGE), which stimulated the secretion of MCP-1 in MCs alone or combined with high concentrations of glucose \[^{25,26}\].

Hyperglycemic condition stimulated MCP-1 production and excretion into the urine \[^{27,28}\]. Patients with type 2 diabetes excrete high levels of MCP-1 in the urine, which correlates with albuminuria, and macroalbuminuric patients with diabetes had higher MCP-1 levels compared with micro or normoalbuminuric patients \[^{29-31}\]. While one study carried out in Korea reported that there was no relationship between MCP-1 promoter SNP and diabetic end-stage renal disease \[^{32}\].

One study agreement with our study which explain that acetyl-11-keto--boswellic acid (AKBA) derivative product led to a significant down regulation of several NF-B–dependent genes such as MCP-1, MCP-3, IL-1, MIP-2, VEGF, and TF\[^{33}\].

CONCLUSION

*Boswellia Carterii* can be consider as good treatment option for reducing glucose and, HbA1c level in diabetes patients after using low dose from it but we need to elevate the dose to effect on immune marker parameter (MCP1) level.

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ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq
CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES


