BIOCHEMICAL EVALUATION OF ANTIHYPERGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *MATRICARIA CHAMOMILLA* LEAVE EXTRACT STUDIED IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract—The hypoglycemic and antioxidative effects of *Matricaria chamomilla* leave extract were evaluated in Streptozotocin (STZ)-induced diabetic rats. The water extract of *Matricaria chamomilla* leave at a concentration of 100 mg/kg body weight/rat/day was orally administered to STZ-induced diabetic rats for a period of 21 days. The elevated levels of blood glucose in the diabetic rats reverted back to near normal after treatment with the water extract. Similarly significant decrease in the levels of serum insulin and C-peptide were elevated to near normal after treatment with water extract, suggesting the antihyperglycemic effect of *Matricaria chamomilla* leave. Determination of thiobarbituric acid reactive substance (TBARS), and both serum liver enzymatic and nonenzymatic antioxidants evidenced the antioxidative potential of the extract, which in turn may be responsible for its hypoglycemic potential. These results indicate that *Matricaria chamomilla* extract effectively reduced the oxidative stress induced by Streptozotocin and potential reduction in blood sugar level.

Keywords—Diabetes, hypoglycemia, oxidative stress, antioxidant enzymes, Streptozotocin, *Matricaria chamomilla*

I. INTRODUCTION

Diabetes mellitus is one of the most pressing global health problems. It is estimated that the prevalence of diabetes mellitus will be more than 300 million in 2025 [1]. Exposure to hyperglycemia for a long time causes oxidative stress and reduces capacities of the endogenous antioxidant defense system which lead to production of several reducing sugars [2]. These reducing sugars can directly react with lipids and increase the production of ROS [3]. The generation of ROS contributes to Streptozotocin (STZ)-induced destruction of pancreatic β-cells [4]. Plants with antidiabetic activities provide important sources for the development of new drugs in the treatment of diabetes. *Matricaria chamomilla* L., known as “chamomile”, has been widely used as an herbal tea all over the world [5]. Chamomile has been used in herbal remedies for thousands of years, known in ancient Egypt. *Matricaria chamomilla* shows different pharmacological activities like anti-inflammatory, anti-cancer, treatment of stress and depression, anti-allergic etc. [6].

In diabetes phytotherapy, the effects of *M. chamomilla* L. have never been demonstrated experimentally in either clinical or experimental diabetes. Thus, in the present study, we investigated the possible antihyperglycemic and antioxidative activities of water extract obtained of *M. chamomilla* L. (MCE) in STZ-induced diabetic rats.

II. MATERIALS AND METHODS

Leaves of chamomile were obtained from the local herbal market of Kingdom Saudi Arabia. Voucher specimens from plant material were deposited at the Herbal Museum, Department of Pharmacology, Faculty of Science, King Abdulaziz University of Medical Sciences for identification.

A. Preparation of the plant sample

The fresh leaves of plant material (5 g) were soaked in 50 ml of boiled water, after 1 h stirring, at room temperature. The supernatant was decanted and the residue was macerated two more days with distilled water. The pooled supernatants were combined and filtered.

B. Chemicals

Streptozotocin was purchased from Fluka (Germany). Hydrogen peroxide, glutathione (GSH), thiolbarbituric acid, phosphate buffer; disodium hydrogen phosphate, sodium dihydrogen phosphate, phenylenediamine, sodium azide, 1-chloro-2,4-dinitrobenzene, sodium nitroprusside, 2,4-dinitrophenylhydrazine, ethanol, hexane, sodium nitrite,
sodium nitrate, sulfanilamide, N-(1-Naphthyl) ethylenediamine dihydrochloride, and vanadium (III) chloride were purchased from Sigma (Germany). All other chemicals and reagents used in this study were of analytical grade.

C. Animals and treatment

Adult male albino rats were selected for the study. They were of the same age (2 months) and weight (150 to 200 g). The animals were housed in acrylic cages in standard conditions of temperature prior to the experiments for 1 week in order to adapt to the laboratory condition, fed with commercial diet and water ad libitum. The principles of laboratory animal care were followed throughout the duration of experiment and instruction given by King Abdulaziz University ethical committee was followed regarding experimental treatments.

D. Experimental design and treatment schedule

The experiment was carried out on 4 groups of five rats in each group to study the effect of plant water extract on STZ-induced diabetes and changes in antioxidants as follows:

- **Group 1:** Healthy control rats, received distilled water.
- **Group 2:** Diabetic control rats. A freshly prepared solution of Streptozotocin or STZ (45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) was injected intraperitonially to overnight fasted rats. STZ injected animals exhibited hyperglycemia within 48 to 36 h [7]. The rats having fasting blood glucose (FBG) values of 250 mg/dl or above were considered for the study.
- **Group 3:** Normal rats administered water extract of chamomile, orally at a dose of 100 mg/kg body weight, and
- **Group 4:** Diabetic treatment rats received 1 ml water extract of chamomile for 21 days after 36 h STZ injection. The treatment with chamomile 100 mg/kg/day [8] was given daily for a period of 3 weeks using gastric cannula [9].

Starting from the 1st day (3rd day of STZ-injection) of extract administration to diabetic rats, FBG (blood glucose) level was measured in every 7th day using glucometer [10]. On the 21th day of extract administration, all the animals were anesthetized (Nesdonal 50 mg/kg, i.p.), blood samples were obtained from hearts of overnight fasted rats by using micro-capillary technique and allowed to clot for 20 min in laboratory temperature and then centrifuged at 10000 rpm for 10 min for serum separation.

E. Biochemical parameters

Serum insulin (Awareness Technologies, USA) and C-peptide (Packard, USA) levels were determined by the ELISA and RIA methods, respectively. The levels of oxidative stress makers including serum malondialdehyde (MDA) as the marker of lipid peroxidation by thiobarbituric acid (TBA) test and nitric oxide (NO) were measured by the method of Thayer [11] and Ding et al. [12], respectively. The levels of reactive oxygen species controlled by antioxidant enzymes like GST, CAT and GPx were measured by the method of Habig et al. [13], Aebi [14] and Rotruck et al. [15], respectively. The nonenzymatic scavengers such as reduced glutathione (GSH) and vitamin C were measured by the methods of Moron et al. [16] and Aye Kyaw [17], respectively.

III. Statistical analysis

The results were expressed as mean ± standard deviation (SD). The data were subjected to one-way analyses of variance (ANOVA) and student’s t-tests using the Statistical Analysis System (SPSS 15.0) program. For all analyses, p values ≤ 0.05 were considered significant.

IV. RESULTS

Figure 1 shows the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats was decreased as compared to control rats.

![Fig. (1): Body weight changes in normal and experimental animals in each group at the initial and final days after treatment.](image-url)

Table 1 illustrates the effect of chamomile on serum glucose, insulin and C-peptide in normal and experimental animals. Glucose levels were found to be significantly increased after STZ administration, and there after decreased by administration of chamomile extract. Decrease in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ. Administration of chamomile extract produced a significant (p<0.01) decrease in the blood glucose as compared to diabetic control. The levels of serum insulin and C-peptide were significantly decreased in diabetic rats when compared with normal rats. Administration of chamomile extract to diabetic rats significantly reversed all these changes to near normal levels.
Table (1): Effect of chamomile on changes in FBG, serum insulin and C-peptide of normal and experimental rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>FBG (mg/dl)</th>
<th>Insulin (µU/ml)</th>
<th>C-peptide (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>126.4±20</td>
<td>13.4±0.72</td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>329±122a</td>
<td>4.5±0.29a</td>
<td>1.6±0.05a</td>
</tr>
<tr>
<td>Normal+chamomile (100mg/kg)</td>
<td>117±19b</td>
<td>12.55±0.19b</td>
<td>4.7±0.21b</td>
</tr>
<tr>
<td>Treated group</td>
<td>131±79a</td>
<td>11.6±0.24b</td>
<td>3.78±0.076b</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD from five rats in each group. *P<0.001 vs. healthy control, **P<0.001 vs diabetic control.

Effect of chamomile on serum oxidative stress markers

Diabetes significantly increased serum malondialdehyde (MDA) - marker of lipid peroxidation- and NO in comparison with the control group. Treatment of diabetic animals with 100 mg/kg/day chamomile extracts significantly inhibited increase of MDA in comparison with the untreated diabetic animals (p < 0.05). These treatments can maintain the level of MDA at the same level compared to that of the control group. The present result revealed that water extracts of chamomile was capable of suppressing NO activity (Table 2).

Table (2): Effect of chamomile on oxidative stress markers in serum of control, diabetic and treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA(nmol/ml)</th>
<th>NO (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>4.42±0.56</td>
<td>48.3±3.8</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>9.59±0.64a</td>
<td>80.09±3.6a</td>
</tr>
<tr>
<td>Normal+chamomile (100mg/kg)</td>
<td>3.89±0.66a</td>
<td>49.17±3.82a</td>
</tr>
<tr>
<td>Treated group</td>
<td>4.24±0.31a</td>
<td>44.12±2.7a</td>
</tr>
</tbody>
</table>

Values are mean ± SD from five rats in each group. *P<0.001 vs. healthy control, **P<0.001 vs diabetic control.

Effect of chamomile on enzymatic and nonenzymatic antioxidants

The enzymatic antioxidant GST, CAT and GPx levels were found to be lower in diabetic rats compared to that of the control rats. These enzymatic antioxidant levels in diabetic rats treated with chamomile extract significantly (p < 0.05) increased to a level closer to the normal values (Table 3). Figure 2 shows the low levels of nonenzymatic antioxidant vitamin C and reduced glutathione were observed in diabetic rats, when compared to that of control rats. The levels of these antioxidants were significantly increased in diabetic rats by treating with chamomile.

Table (3): Effect of chamomile on enzymatic antioxidants in serum of control, diabetic and treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GST (U/ml)</th>
<th>CAT (U/ml)</th>
<th>GPx (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>46.8±1.05a</td>
<td>21.98±1.33</td>
<td>77.09±1.25</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>20.39±0.8a</td>
<td>8.51±0.09a</td>
<td>13±0.4a</td>
</tr>
<tr>
<td>Normal+extract (200mg/kg)</td>
<td>46.9±0.64a</td>
<td>21.07±2.05a</td>
<td>75±1.43a</td>
</tr>
<tr>
<td>Treated group</td>
<td>41.06±1.33</td>
<td>19.73±0.63</td>
<td>68.24±1.63a</td>
</tr>
</tbody>
</table>

Values are mean ± SD from five rats in each group. *P<0.001 vs. healthy control, **P<0.001 vs diabetic control.

V. DISCUSSION

Diabetes mellitus (DM) is an endocrine disorder characterized by chronic hyperglycemia with many disturbances of carbohydrate, fat, and protein metabolism due to decrease in insulin secretion, insulin action, or both. Hyperglycemia is a main cause of the oxidative stress in diabetic patients and reduces the capacity of the endogenous antioxidant defense system [3]. Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. The underlying mechanism(s) of this blood glucose lowering activity may be the stimulation of peripheral glucose utilization, especially in muscle and adipose tissue, and/or the restoration of enzyme activity which play a part in the glucose and glycogen metabolism.

Increasing blood glucose levels in diabetes leads to overproduction of free radicals, defined as an imbalance between oxidants and antioxidants. Glucose autooxidizes in the presence of transition metal ions generating oxygen-free radicals make the membrane vulnerable to oxidative damage [18]. Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and are secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion than insulin alone [19]. In the present study, treatment with chamomile showed significant increase in serum insulin and C-peptide levels in diabetic rats. These results indirectly indicate that part of the antihyperglycemic activity of this plant is through increasing the release of insulin from the pancreas.

This study showed that STZ group exhibited higher values of cellular lipid peroxidation which measured through the
malonaldehyde level (MDA) and a decrease the nonenzymatic antioxidants (levels of ascorbic acid and GSH) in diabetic rats, as compared to normal rats. GST, CAT, and GPX levels in the serum of chamomile treated rats were found to be significantly higher than the STZ group. These results are in agreement with the previous studies which reported that the reason of chamomile usage in treatment of liver diseases is in its efficacy in inhibition of reactive oxygen species and lipid peroxidation [20]. The decrease of nonenzymatic antioxidants levels might be due to increased utilization for scavenging free radicals. The treatment of STZ-induced diabetic rats with chamomile for 21 days resulted in a marked decrease in the MDA and increase in the ascorbic acid and GSH levels. The cause of the increase in the nonenzymatic antioxidants levels in treated diabetic rats might be due to decreased utilization, as lipid peroxidation is low. Decreased activities of enzymatic antioxidants such as GST and CAT have been well documented in STZ-induced diabetic rats [21]. The present study revealed the decreased activity of serum GST and CAT in diabetic rats, as reported previously, which could be due to increased consumption for free radicals' detoxification.

Nitric oxide synthase (NOS) catalyzes the production of nitric oxide (NO). Inducible nitric oxide synthase (iNOS) is expressed by vascular endothelial cells and smooth muscle cells in response to cytokines, unlike the two other types of NOS, which are constitutive. NO produced by iNOS is implicated in inflammatory diseases [22]. Food and phytochemicals exert NO-suppressing activity via three different pathways: The blocking of iNOS expression, inactivation of iNOS catalytic function and the scavenging NO; while NO suppressing effect primarily through regulation of cellular iNOS expression. Moreover, Aslan et al. [23] demonstrated the suppression of the oxidative stress and inflammatory response related to diabetes through the inhibition of tumor necrosis factor –α- (TNF-α) signaling. Natural triterpenes such as ginsenosid Rh1, triterpenes isolated from Panax ginseng, inhibited iNOS expression and the activation of NF-α. In addition, oleanolic acid glycoside isolated from ampelopsis radix, markedly suppressed the activity of TNF-α and production of NO.

In conclusion, the results of this study represent that the administration of MCE showed antihyperglycemic effect, which controls the blood glucose level and, thereby, inhibits the formation of free radicals or it may scavenge the reactive oxygen metabolites through various antioxidant compounds in them. On the other hand, MCE has a favorable effect to inhibit the histopathological changes of the pancreas in STZ-induced diabetes. Therefore, M. chamomilla L. may provide new alternatives for the clinical management of diabetes and the consumption of M. chamomilla L. aerial part can prevent the complications of hyperglycemia associated with diabetes. Further studies will be needed to determine the active components in MCE and their role in controlling diabetes, and to reveal the exact underlying mechanism(s) on how the MCE can treat diabetes.

**ACKNOWLEDGMENT**

This work was supported by research Grant from University of King Abdulaziz, Jeddah.

**VI. REFERENCES**


