Effect of *Lactuca serriola* on β-Cell dysfunction and Glucose Tolerance induced by High Sucrose fed in Albino Rats

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**Abstract** Herbal remedy has been used as efficacious choice for treating type 2 diabetes mellitus in last two decades. The aim of current study is to investigate the hypoglycemic action of *Lactuca serriola* on chronic hyperglycemia model in male rats. The experiment included four groups and five rates were applied for each group. All rates (except control group) treated with 60% sucrose in diet for 8 weeks. The first group was considered as high sucrose feed (HSF) or model, whiles the second and third groups where administrated with 200 and 500 mg/Kg BW. of *Lactuca serriola* by gastric gavage respectively. Hyperglycemia was assessed by evaluating fasting blood sugar, serum insulin, HbA1c, oral glucose tolerance test, HOMA-IR and HOMA-B. Moreover, lipid profiles were also measured. The results indicated that induction of chronic hyperglycemia model for 8 weeks led to β-cell dysfunction as HOMA-B significantly (P<0.05) decreased, while both doses of *Lactuca serriola* restored β-cell function and insulin secretion. Furthermore, *Lactuca serriola* improved glucose tolerance significantly via measuring the total area under the curve. This finding suggested that *Lactuca serriola* could be applied as a therapy for chronic hyperglycemia.

**Key words**: *Lactuca serriola*, hyperglycemia, Insulin, rats

**Introduction**

Chronic hyperglycemia is an endocrine related metabolic disorder which enhanced by Chronic exposure of the subject to high sucrose feeding (HSF) [1]. This disorder is indicated by high blood glucose level (hyperglycemia), high serum lipid concentration (hypercholesterolemia), high blood pressure (hypertension), and cardiovascular disease as a result of alterations produced in carbohydrate, lipid, and protein metabolism [2]. It has been proved that chronic exposure of pancreatic β-cells to high glucose may lead to pancreatic β-cell dysfunction and glucotoxicity which characterized by an increase in apoptosis and a marked deficit of glucose-stimulated insulin secretion (GSIS) [3, 4].

The effects of dietary carbohydrates, particularly sucrose, on food intake and body weight gain have been tested extensively in experimental animals [5, 6]. There are reasonable mechanism and research evidence that supports the suggestion that consumption of excess sugar promotes the development of cardiovascular disease and type2 diabetes [7]. Malik et al in 2010 reported that daily consumption of...
sugar-sweetened beverages was associated with 15% increase risk for development of Type2 diabetes [8].

Traditional herbal medicines are used throughout the world for the curative of various types of diseases, including diabetes [9]. Therefore uses of medicinal plants to treat hypoglycemia and hyperglycemia cases are reasonable interest for ethno botanic community [10].

This has led to the rising search for plants with medicinal use. The search for plants with medicinal use has led to some ethnobotanical studies that have documented traditional medicinal plant species, the mode of preparation and uses by local communities in some parts of the country [11, 12]. *Lactuca serriola* is one of herbal plant from family (Asteraceae) which includes approximately 100 species and is distributed in temperate and warm regions of the Northern hemisphere, as well as Northern Africa and North and South- America [13, 14].

This plant can create a basis for phytochemical evaluation which can lead to the discovery of biologically active compounds that can be used as starting materials in the development of new drugs targeting selected diseases such as diabetes mellitus and as anti-inflammatory [15].

In traditional medicine, different properties have been reported for *Lactuca serriola*, such as antitussive, expectorant, vaso relaxant, purgative, and antiseptic properties [16]. *Lactuca serriola* has been reported also to decrease reactive oxygen species (ROS) production and lipid peroxidation [17].

Studies have been shown that the methanol extracts of *Lactuca serriola* contain about 400 µgm of phenolic acid, So that phenolic antioxidants have also been shown to delay the development of chronic diseases such as cardiovascular diseases and Alzheimer’s disease [18]. Further studies reveals that the plant contains lactucopicrin, lactucin, lactucone, vitamins A, B1, B2, and beta carotene and iron [19]. It has been demonstrated that the hydroalcoholic extract of *Lactuca serriola* had the hypoglycemic effects in Streptozotocin (STZ) induced diabetic male rats [15].

Several investigations have been conducted for applying local Iraqi herbs as anti-diabetic, anti-hyperlipidemia and anti-cancer therapy. The current attempt is the extension of these researches that applies *Lactuca serriola* as anti-hyperglycemic and anti-diabetic herbal treatment in sucrose induced chronic hyperglycemia experimental model in male rats.

**Materials and methods**

**Plant collection**

Fresh Leaves of *Lactuca serriola* were collected in Mountains of rawanduz City around the end of April 2018. The plant species was identified taxonomist in agriculture dept. in Salahaddin university-Erbil. The Leaves were dried under shadow at room temperature and stored in plastic package until use.

**Animals Experiment**

Adult Male Albino rats (180-210g) were used for the study; all animals were exposed to a 12-hour light/dark cycle at 25±2°C and were given free access to tap water ad libitum daily. Twenty rats were divided into 4 groups (n=5) including control group. Sucrose feeding group receiving 60% sucrose in diet for 8 weeks, and they were divided to three groups. The first group regarded as the model group which they were treated with 60% sucrose alone, while the two other sucrose feeding groups were given aqueous extract of *Lactuca serriola* leaves at doses of 200 and 500 mg/kg BW by stomach gavage once daily along with sucrose.
Preparation of aqueous extract of *Lactuca serriola* leaves

Dried *Lactuca serriola* leaves were powdered; Fine powder (50g) of leaves was macerated with 250ml boiled distilled water for 12hr. and was filtered with filter paper. The extract was evaporated in rotary evaporator at 45-55 °C.

Serum Biochemical parameters

Rats were fasted overnight and anesthetized using (Xylazine/Ketamine). Blood sample were collected after excision of the heart and serum was separated by centrifugation and stored at -40 °C. Serum glucose were determined using glucose monitoring meter (ACCU-CHEK Glucometer, Germany), Blood HbA1c% was measured by COBAS INTEGRA 400 plus (Roche Diagnostics), serum insulin levels were assessed using SunLong Biotech Rat Insulin (INS) ELISA Kit( SL0373Ra). IR-HOMA and HOMA-B (β-cell function) were evaluated using homeostatic model assessment (HOMA) index: HOMA-IR and HOMA_ β were calculated using the following equations [20].

\[
\text{HOMA-IR} = \frac{\text{Insulin (U/ml)} \times \text{glucose (mmol/L)}}{22.5}
\]

\[
\text{HOMA_ β} = 20 \times \frac{\text{Insulin (U/ml)}}{\text{glucose (mmol/L)}} - 3.5
\]

Lipid profiles including serum total cholesterol (TC) serum triglycerides (TG) and High density lipoprotein were measured by (COBAS INTEGRA 400 plus), in addition to Non-HDL cholesterol using the following equation [18].

Non HDL cholesterol = Serum Total cholesterol - Serum HDL-C

Data analysis

All data were analyzed using Graphpad prism 6.01. Descriptive data were expressed as mean ± SEM. Comparison between means were performed by tukey’s post hoc analysis after one-way ANOVA. P<0.05 was considered as significant.

Results

Oral administration of *Lactuca serriola* extracts significantly (p<0.0001) improved fasting blood glucose level when compared with HSF or model group. This reduction was not significant when compared with the control group which indicates the ability of both doses to restore blood glucose to the normal level, as shown in figure 1a.

Figure1b recorded significant differences among the treatments (P<0.05) regarding serum HbA1c% level. High sucrose feeding didn’t able to induce hemoglobin glycation (HbA1c) to a significant level comparing to control rats, while the treatment of rats with high doses (500mg/kg bw) plant extracts significantly declined HbA1c compared to HSF rats.
Figure 1: Anti-diabetic action of aqueous extract *Lactuca serriola* on fasting blood sugar and HbA1c in high sucrose feeding rats. (a) The level of blood glucose showed significant difference between model group with all other groups. (b) presents the level of HbA1c which showed non-significant difference between the groups, except the difference between model and high dose of plant. All the data represented as mean ± S.E (N=5) and one-way ANOVA and Tukey’s post-hoc were performed for compassion among them. **** indicates (P<0.0001), *** indicates (P<0.001), and * indicates (P<0.05).

Measuring total area under the curve (AUC) for comparison between the treatments regarding glucose tolerance recorded significant reduction of glucose under the action of plant extract versus model group (figure 2).

Figure 2 Oral glucose tolerance curve under the action of aqueous extract *Lactuca serriola* 200 and 500 mg/Kg BW in high sucrose feeding rats. The total area under the curve showed highly significant areas among all the groups. All the data represented as mean ± S.E for the total area under the curve (AUC) (N=5) and one-way ANOVA and Tukey’s post-hoc were performed for compassion among them.

The current results of the serum insulin level demonstrated significant differences among the treatments (P<0.0001). The high sucrose diet led to declining of insulin significantly comparing to control rats, while the treatment of rats with both doses of plant extracts restored the level of insulin significantly compared to model as shown in figure 3.
Figure 3 Effect of aqueous extract *Lactuca serriola* 200 and 500 mg/Kg BW on insulin levels in high sucrose feeding rats. The level of serum insulin level showed significant difference between all groups. All the data represented as mean ± S.E (N=5) and one-way ANOVA and Tukey’s post-hoc were performed for compassion among them. **** indicates (P<0.0001).

Evaluation the degrees of HOMA-IR illustrated that insulin resistance was declined significantly (P<0.05) in model group compared to control as a result of decreasing in the serum insulin level, while the plant extract doses restored this declining as shown in figure 4a. Whereas, HSF rats recorded significant β cell dysfunction as it reported significant reduction in HOMA- β ratio comparing to control, and a clear improving in the β cell function was detected via administration of *Lactuca serriola* which was dose dependent (Figure 4b).

Figure 4 Effect of aqueous extract *Lactuca serriola* 200 and 500 mg/Kg BW on HOMA-IR and HOMA-β in high sucrose feeding rats. (a) The level of HOMA-IR showed restoring of the level of HOMA-IR in 200mg/kg bw plant extract. (b) presents the level of HOMA-β which showed severe significant reduction in model group, and dose dependent improving of β cell function. All the data represented as mean ± S.E (N=5) and one-way ANOVA and Tukey’s post-hoc were performed for compassion among them. **** indicates (P<0.0001), *** indicates (P<0.001), ** indicates (P<0.01) and * indicates (P<0.05).
The data analysis in table 1 showed significant differences among the treatments (P<0.0001) regarding cholesterol level. HSF rats showed significant (73.8±1.15) comparing to control rats (59.2±2.51), while only high dose (500mg/kg bw) of plant extracts was able to reduce serum cholesterol significantly compared to model. The results in table 1 also confirmed the priority of Non-HDL cholesterol on serum total cholesterol. Both Lactuca serriola extracts doses recorded highly significant decreasing of bad cholesterol comparing to HSF rats. A dramatic falling in HDL-C level was found in sucrose feeding rats (P<0.001), while the treatments of rats with both doses of extracts restored the level to even more than control group significantly.

The serum TG level was increased significantly in HSF rats, and the anti-hyperglycemic effect of both dose Lactuca serriola extracts were demonstrated as they showed significant declining in TG level comparing to HSF rats.

Table 1. Serum lipid profile measures in high sucrose feeding rats treated with 200 mg/kg bw and 500mg/kg bw Lactuca serriola aqueous extract.

| Lipid Profile Variable | control Mean ± S.E | model Mean ± S.E | 200mg Mean ± S.E | 500mg Mean ± S.E | Significant (P value) |
|------------------------|--------------------|------------------|------------------|------------------|-----------------------|
| Non HDL cholesterol    | 40.2 ± 2.83        | 57.6 ± 2.92      | 36.84 ± 2.50     | 26.6 ± 2.83      | ****                 |
| LDL-C                  | 28.2 ± 2.26        | 35 ± 1.14        | 32.2 ± 0.86      | 31.4 ± 2.42      | N.S.                 |
| HDL-C                  | 19 ± 1.58          | 16.2 ± 2.26      | 30.16 ± 0.66     | 35 ± 1.73        | ****                 |
| Total Cholesterol      | 59.2 ± 2.51        | 73.8 ± 1.15      | 67 ± 2.34        | 61.6 ± 2.58      | **                   |
| TG                     | 35.4 ± 2.67        | 50.6 ± 4.75      | 33.6 ± 1.32      | 24 ± 0.7         | ****                 |

**** indicates P<0.0001,  ** indicates P<0.01, NS indicates non-significant differences

Discussion

The present study suggested the hypoglycemic and hypolipidemic potency of Lactuca serriola aqueous extract on chronic hyperglycemic experimental rats. In spite of very few studies on Lactuca serriola as anti-diabetic herb, our investigation considered as the first attempt of Lactuca serriola action on chronic hyperglycemia experimental model.

High sucrose feeding rat were characterized with high fasting blood sugar, HbA1c and impaired glucose tolerance test. Several previous literatures proved glucose elevation in this model [21] and this was likely due to reduce insulin level and increase glucose ingestion [19]. While oral administration of Lactuca serriola extracts improved blood glucose, HbA1c, glucose tolerance in HSF, in addition to restoring the level of insulin significantly. The therapeutic activity of Lactuca serriola could be related to the antioxidant scavenging capacity of the polyphenol and other components found within the plant [22]. Our results was agree with (Janni et al., 2016) who conclude that hydroalcoholic extract of Lactuca serriola reduces blood glucose level in Streptozotocin-induced diabetic rats [12].

Type 2 diabetes mellitus (DM) is caused by both insulin resistance (IR) and β-cell dysfunction each of which is independently associated with the onset of DM [23, 24]. HSF rats in our results were characterized with β-cell dysfunction but not insulin resistance. The test of insulin resistance (HOMA–IR) decreased in HSF rats which indicated that 8 weeks of chronic hyperglycemia is not enough for induction of type 2 DM. The improvement of β-cell function by the extract may due to the antioxidant and anti-inflammatory activity of many substances such as oxazolidine which prevent β-cell from inflammation and destruction [22].

The hypeplipidemia produced by HSF may be due to increased accumulation of triglyceride and cholesterol in blood which decreased catabolism of the rats [25]. Administration of HSF rats to Lactuca serriola extracts significantly improved the triglyceride elevation at both doses.
This finding, for the first time, showed that the doses of 200 and 500 mg/kg of aqueous extract of Lactuca serriola has therapeutic actions on blood sugar, oral glucose tolerance, HbA1c, serum Insulin, triglyceride and serum cholesterol and its derivatives in sucrose induced chronic hyperglycemia experimental model in male rats.

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