Plant Archives Vol. 21, Supplement 1, 2021 pp. 1654-1657 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.260

TREATMENT OF INDUCED DIABETES IN RATS BY NANOSILYMARIN

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ABSTRACT

In the present study, the mean differences of some parameters according to study groups including (Control group, diabetic rats, diabetic rats with Nano silymarin, Nano silymarin and Diabetic rats with extract of silymarin) were studied. The mean of diabetic rats groups with Nano silymarin was more decrease in sugar (mg/dl) when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was less decrease than groups with Nano silymarin. The mean of insulin in diabetic rats groups with Nano silymarin was increased when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was no effected with groups diabetic rats treated with Nano silymarin. The mean of IGF in diabetic rats groups with Nano silymarin was decreased when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was more decrease than groups with Nano silymarin. The mean of HOMA Insulin resistance in diabetic rats groups with Nano silymarin was decreased when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was more decrease than groups with Nano silymarin. The mean of protein in diabetic rats groups with Nano silymarin was not effected when compared with diabetic rats groups, also when compared diabetic rats groups with extract silymarin, the mean was not affected. However, there was significant differences between means of albumin according to study group. The mean of cholesterol and Triglyceride in diabetic rats groups with Nano silymarin was decreased when compared with diabeticrats groups, while when compared diabetic rats groups with extract silymarin, the mean was mild decrease than groups with Nano silymarin. High density lipoprotein (HDL) in diabetic rats groups with Nano silymarin was increased when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was mild increasedthan groups with Nano silymarin. In addition, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in diabetic rats groups with Nano silymarin was decreased when compared with diabetic rats groups, while when compared diabetic rats groups with extract silymarin, the mean was mild decreased than groups with Nano silymarin. This study aims to compare between Silybum marianum extract and Nano- particles activity in treatment of diabetes mellitus.

***Keywords*:** Silybum marianum extract, diabetes mellitus, insulin, insulin-like growth factor.

Introduction

Diabetes mellitus (DM) is the metabolic disease, which effect on metabolism of carbohydrate, lipid, and protein. It is expressed precisely by persistent hyperglycemia, resulting from defects in insulin secretion, insulin action or summation of both, impaired secretion and wrong action (Alves *et al*., 2013). Nanotechnology is the study of extremely small structures, having size of 0.1 to 100 nm. Nano medicine is a relatively new field of science and technology (Barbetti & D'Annunzio, 2018). Silymarin (Sm) is a polyphenolic component extracted from *Silybum marianum*(Abenavoli *et al*., 2018). A biomarker is a valuable tool due to the possibility to distinguish two or more biological states from one another, working as an indicator of a normal biological process, a pathogenic process or as a reaction to a pharmaceutical intervention (Strimbu & Tavel, 2010). Insulin is a peptide hormone produced and secreted by β-cells of islets of Langerhans of the pancreas (Chatterjea, 2012). Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a hormone similar in molecular structure to insulin which plays an important role in childhood growth, and has anabolic effects in adults (Höppener *et al*., 198). Lipids are a group of fats and fat-like substances that are important constituents of cells and sources of energy (Ahmad, 2015). Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues (Chang *et al*., 2019). Albumin is a family of globular proteins, the most common of which are the serum albumins. All the proteins of the albumin family are water-soluble, moderately soluble in concentrated salt solutions, and experience heat denaturation (Douglas *et al*., 2019).

Materials and Methods

The present study was completed in the laboratory of Clinical Biochemistry Department in College, of Veterinary Medicine, / University of Al-Qasim Green. The collection of samples was directed during the period from (1st of January 2020) until (30th of March 2020).

Study Groups

The study groups of the present study were (75) rats of diabetic and controls, which were divided into five groups according to variation between groups.

Preparation of animals

A total seventy five Mature Wister albino rats (75 male) with a mean weight of 180 ± 20 g, obtained from the animal house at the College of Veterinary Medicine, Al Qasim Green University. All animals were kept in isolated room and maintained on controlled conditions (temperature 20-25 ºC, humidity 30-70% and alternating light and dark 12h dark/light cycle).

Induction of Diabetes

Diabetes was induced in diabetic and diabetic treated groups by a single intra-peritonealy injection of alloxan monohydrate (150mg/kg of body weight), (sima chemical Co., USA), freshly dissolved in 5 sterile normal saline. The rats were fasted 12hr. before and 12hr. after alloxan injection.

Preparation of silymarin-TPGS nanoparticles

According to (Gauttam and Kalia, 2013).

Determination of Fasting Serum Glucose Concentration

According to (Chao *et al*., 2003).

Determination of Serum Total Cholesterol

According to (Allain *et al*., 1974).

Determination of Serum HDL-Cholesterol

According to (Warnick, 1995).

Determination of Serum Triglycerides, VLDL-C, LDL-C

According to (Al-gazally *et al*., 2014).

Determination of Serum Insulin, IGF-1 Concentration

According (Hermenean *et al*., 2016).

Statistical Analysis

Statistical analysis was carried out using SPSS version 23. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups.

Results and Discussion

In the present study, in table (1), it was observed that, the mean differences of sugar (mg/dl) according to study groups including (Control group, diabetic rats, diabetic rats with Nano silymarin, Nano silymarin and Diabetic rats with extract of silymarin). The results showed that, there were significant differences between means of sugar according to study group (P<0.001), the mean of diabetic rats groups with Nano silymarin (197.5 ± 12.6) was more decrease when compared with diabetic rats groups (269.7 ± 29.7), while when compared diabetic rats groups with extract silymarin, the mean was less decrease (220.2 ± 14.2) than groups with Nano silymarin. These results were agreement with results obtained by (El-Far *et al*., 2014) who found that, blood glucose levels, There was significant change in the mean serum level of glucose in control treated with Silymarin (100 mg/kg body weight) when compared with normal control group (*P*>0.05). A highly significant increase in blood glucose level is observed in diabetic control rats compared with normal control rats, while, a highly significant decrease in the blood glucose level is observed in diabetic rats-treated with Silymarin (100 mg/kg body weight) as compared to the diabetic control group. The effect of silymarin on level of glucose has anti-inflammatory and antioxidant properties with is improve the function of beta cell to produce insulin and reduce insulin resistance a free radical scavenger which prevent lipo-pre-oxidant ion on the pancreatic activity of superoxide dismutase and glutathione invert induced diabetic by alloxan. There were significant differences between means of insulin according to study group. (P=0.009), the mean of diabetic rats groups with Nano silymarin (9.0 ± 2.0) was increased when compared with diabetic rats groups (7.9 ± 2.4), while when compared diabetic rats groups with extract silymarin, the mean was no effected (9.6 ± 2.0) with groups diabetic rats treated with Nano silymarin. This indicated that, the Nano and extract Silymarin were increased released of insulin in same effect. The results in this study were agreement with results obtained by(Soto*et al*., 2010), who found that, Silymarin can increase serum insulin, reduce serum glucose and rise of antioxidant enzymes, as well as recover endocrine function and pancreatic morphology in diabetic animals.There were significant differences between means of IGF according to study group. (P<0.001). the mean of diabetic rats groups with Nano silymarin (204.5 ± 29.5) was decreased when compared with diabetic rats groups (170.1 ± 30.5), while when compared diabetic rats groups with extract silymarin, the mean was more decrease (192.8 ± 20.1) than groups with Nano silymarin. This indicated that, the Nano Silymarin was regulated of released of insulin growth factor more than extract silymarin. The Effect of silymarin on IGF-1 was acted by modulation the activity and function of protein and receptor such as IGF-1. The results in this study were identical with results obtained by (Shaker *et al*., 2011)who found that Serum levels of IGF-I were decreased, in diabetic rats compared with controls, while disagreement with results obtained by (Caceres-Corte*set al*., 2012) who found that, Serum IGF-I levels were found to decrease from (577.2 ng/ml) to (253.0 ng/ml) after Silymarin injected (p<0.005). There was significant differences between means of HOMA Insulin resistance according to study group. (P<0.001). the mean of diabetic rats groups with Nano silymarin (4.35 ± 1.04) was decreased when compared with diabetic rats groups (4.91 ± 1.47), while when compared diabetic rats groups with extract silymarin, the mean was more decrease (5.15 ± 1.06) than groups with Nano silymarin. This indicated that, the extract Silymarin was regulated of released of insulin resistance more than Nano silymarin. These results were agreement with results obtained by (Cheng*et al*., 2014) who found that, administration of Silymarin produced decrease in insulin resistance in the diabetic rats. Silymarin was administered to diabetic rats, due to decrease of induce insulin resistance. Therefore, No intolerance, side effects, or allergic reactions were observed(Croz*et al*., 2013).However, the results in this study were identical with results of (Long *et al*., 2012) who found that, the insulin resistance was significantly increased in diabetic rats induced, whereas the glucose levels were decreased in diabetic rats with Nano silymarin. There was not significant differences between means of protein according to study group (P=0.767). the mean of diabetic rats groups with Nano silymarin (7.59 ± 0.58) was not effected when compared with diabetic rats groups (7.66 ± 0.66), also when compared diabetic rats groups with extract silymarin, the mean was not effected (7.58 ± 0.59). However, there was significant differences between means of albumin according to study group (P=0.002).The presence of albumin in blood is considered as indicator or raring signal to diabetic, albumin does not necessary reflect on diabetic, so, there is a need to find biomarkers that help in identification of patients risk of

the disease and monitoring preventive and therapeutic effect. Albumin hearty glaciated with diabetic, the decrease of albumin level was effected on protein glycation and glycosylated hemoglobin which is measure high glucose level. There was significant differences between means of lipid profile according to study group (P<0.001). the mean of cholesterol and Triglyceride in diabetic rats groups with Nano silymarin (199.73±10.60;185.20±12.67) was decreased when compared with diabetic rats groups (208.40 ±12.52, 205.07±12.95), while when compared diabetic rats groups with extract silymarin, the mean was mild decrease (202.40 ±9.65, 195.27±12.74) than groups with Nano silymarin. However, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was studied, it was found that the mean of high density lipoprotein (HDL) in diabetic rats groups with Nano silymarin (48.20 ± 5.24) was increased when compared with diabetic rats groups (39.07 ± 6.53), while when compared diabetic rats groups with extract silymarin, the mean was mild increased (44.20 ± 4.14) than groups with Nano silymarin. In addition to that, the mean of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in diabetic rats groups with Nano silymarin (114.46 ±13.788; 37.04 ± 2.53) was decreased when compared with diabetic rats groups (127.21±17.397; 41.08 ± 2.52), while when compared diabetic rats groups with extract silymarin, the mean was mild decreased (119.22 ±8.44; 38.82 ± 2.20) than groups with Nano silymarin. These results were agreement with results obtained by (Pavan Kumar, 2012) who found that, The rats fed on high cholesterol diet showed significant increase in serum total cholesterol, Triglycerides, LDL-C and VLDL-C, when treatment with Nano silymarin, significantly decreased serum total cholesterol, Triglycerides, LDL, in addition to that, rats treated with Nano silymarin showed significant increase in hepatic HDL and decrease in other lipid profiles.Silymarin was effected on lipid profile in diabetic rats by decrease cholesterol and LDL, while in normal condition the cholesterol was reduced absorption of cholesterol from intestine, increase HDL and decrease liver content by inhibit the enzyme, which is important in synthesis of cholesterol (Ubaid, 2017).

Table 1 : Mean differences of parameters according to study groups

Marker	Study groups					P value
	Control (15)	DR (15)	DR with NS (15)	NS (15)	DR with extract S (15)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Sugar	95.27 ± 9.66	269.7 ± 29.7	197.5 ± 12.6	94.9 ± 6.51	220.2 ± 14.2	<0.001*
insulin	5.5 ± 2.1	7.9 ± 2.4	9.0 ± 2.0	5.2 ± 1.6	9.6 ± 2.0	0.009*
IGF	225.1± 45.7	170.1 ± 30.5	204.5 ± 29.5	261.2 ± 20.1	192.8 ± 20.1	<0.001*
HOMA I.R	1.96 ± 0.48	4.91 ± 1.47	4.35 ± 1.04	1.65 ± 0.39	5.15 ± 1.06	<0.001*
Protein	7.3 5 ± 0.82	7.66 ± 0.66	7.59 ± 0.58	7.49 ± 0.75	7.58 ±0.59	0.767
Albumin	4.34 ± 0.24	4.00 ± 0.37	4.14 ± 0.31	4.44 ± 0.22	4.22 ± 0.33	0.002*
Cholesterol	180.33±10.04	208.40±12.52	199.73±10.60	171.80±12.27	202.40±9.65	<0.001*
Triglyceride	130.20±11.63	205.07±12.95	185.20±12.67	121.73±9.66	195.27±12.74	<0.001*
HDL	53.80 ± 6.73	39.07 ± 6.53	48.20 ± 5.24	52.13 ± 4.17	44.20 ± 4.14	<0.001*
LDL	101.17 ±10.473	127.21±17.397	114.46±13.788	95.28 ±14.02	119.22±8.44	<0.001*
VLDL	26.04±2.32	41.08±2.52	37.04 ±2.53	24.21± 2.03	38.82 ± 2.20	<0.001*

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