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Assessment of the Glucoregulatory Enzymes in induced Diabetic Male Rats treated with silver Nanoparticles of Peel *Raphanus sativus L.* Extract

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Abstract:-

This study includes the investigation of the hypoglycemic effect by assay the blood glucose levels and the glucoregulatory enzymes such as Aldolase A, glucose-6-phosphate dehydrogenase, lactate dehydrogenase and Total antioxidant capacity of silver nanoparticles prepared from peel *Raphanus sativus L* extract in diabetic rats. The experimental groups (30 rats) were equally divided into five groups six rats for each group:

G_1 : control group (treated with normal saline) ,$G_{2:}$ diabetic groups induced by alloxan in a single dose (150 mg/kg b.w),G_3: Diabetic groups (alloxan 150 mg/kg b.w intraperitoneal injection for 60 days of 100ppm of Nanoparticles extract,G_4: preventive group(intraperitoneal injection for 60 days of 100ppm of Nanoparticles + then induces diabetic by alloxan in a single dose (150mg/kg b.w),G_5: Control treated with 100 ppm of nanoparticles for 60 days. The results shown a significant decreased in the levels of blood glucose, glucose-6-phosphate dehydrogenase and lactate dehydrogenase for the treated group(G3) and the preventive group(G_4) when compared with diabetic group while the Aldolase A activity and total Antioxidant capacity (TOAC) were significantly increased for the treated group(G3) and the preventive group(G_4) when compared with diabetic group.

Keywords:

***Raphanus sativus L;* Silver nanoparticles AgNPs, , Alloxan, Diabetes Mellitus, Glucose , Aldolas A ,Glucose-6-phosphate dehydrogenase, lactate dehydrogenase ,Total antioxidant capacity** .

Introduction:-

Nanotechnology provides a long knowledge of applied science and technology to control the matter on the atomic and molecular scale. It is an important and emerging technical tool for development of eco-friendly and reliable methodology for synthesis of nanoscale materials using biological sources (1). Heredity and environmental agents have an important function in diabetes. The disease is

properties by raising blood sugar levels due to the failure in insulin secretion or action. Polyuria, polydipsia, and polyphagia are the symptoms of diabetes and hyperglycemia taller in diabetic condition leads to cardiovascular disease, retinopathy, and nerve defect (2,3). A good control of hyperglycemia known initial on and continuous life long, a particular with diabetes can enjoy a good quality of life and low the risk of these long-term complications, for example cardiovascular disease, blindness, renal failure, amputations and stroke (4).

Type two diabetes (T2DM) a heterogeneous metabolic disease properties
About insulin resistance in peripheral tissues, organized with impaired insulin secretion from pancreatic β-cells (5). The initial fundamental irregularity is a visual (relative or absent) deficit of the hormone insulin.
Fructose–1,6-bisphosphate aldolase (D Fru–1,6-bisphosphate glyceraldehyde–3-P-lyase; FBPA; E.C. 4.1.2.13) catalyses the reversible cleavage of fructose–1,6-bisphosphate (FBP) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde–3-phosphate (GAP) in the glycolytic pathway of prokaryotic and eukaryotic organisms. FBPA is also an essential enzyme for the reversible gluconeogenesis and the Glucose-6-phosphate is converted to fructose-6-phosphate, then further converted to Glucosamine-6-phosphate, which is catalyzed by Glutamine: fructose-6-phosphate aminotransferase (GFAT) yielding Uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc) as an end product. This pathway is essential for the synthesis of glycolipids and glycoproteins glycosyl side chains. UDP-GlcNAc is responsible for flux regulation in this pathway. Increased flux is implicated in β-cell dysfunction in diabetes (6,7).
LDH is a ubiquitous enzyme, which catalyzes the oxidative conversion of the substrate pyruvate to lactate and has been used as an inflammatory marker. LDH is composed of five isoenzymes (LDH1, LDH2, LDH3, LDH4, and LDH5). Lactate oxidation to produce pyruvate by lactate dehydrogenase (8). Under anaerobic conditions lactate is synthesized from pyruvate in a one reaction step by the enzyme lactate dehydrogenase (9).

Antioxidants are chemicals or biological agents capable of neutralizing the potentially damaging action of free radicals. In the diabetic state, free radicals increase can be scavenging by antioxidants. The body system has a well-developed antioxidant defense mechanism that helps to prevent and scavenge free radicals formation, thereby limiting their deleterious effects (10).

Diabetes may be induced by some drugs such as Alloxan and Streptozotocine. Alloxan {(2,4,5,6)tetraoxyhexa hydro pyrimidine} is one of the narrow models

employed to induce diabetes mellitus within the experimental animals. It has been shown to be selectively toxic to duct gland beta cells due to it preferentially accumulates in the β- cells as glucose analogues. Additionally, the cytotoxic action of Alloxan is mediated mainly by the generation of reactive oxygen species (ROS) (11).

The aim of this study to assess the activity of enzymes that related with the glucose levels in diabetic rats to investigate the role of silver nanoparicles of peel Raphanus sativus L extract that acts as a reducing and stabilizing agent in an improvement of these enzyme activities as well as to verify of the antidiabetic effect of these nanoparticles.

Materials and methods:

1-Collection of Plant Samples, Preparation of the Extracts and Synthesis of silver Nanoparticle.

Collection of the plant

Peel of *R. sativus* were collected from local markets, Babylon city – Iraq roots of *Raphanus sativus*. L washed in taped water and then removed the nutshell from roots, dried in shade at room temperature for 7 days, grinding in blender then become powder.

Preparation of *R. sativus* extract

One gram of peel *R. sativus*. L dissolved in 100 ml deionized water at room temperature and shaking by a magnetic stirrer for one hour. The aqueous cold extract was filtered using filter paper. The filtered extract was stored in the refrigerator to use.

Synthesis and Optimization of silver nanoparticles using Peel Extract of *Raphanus sativus* L Extract.

The preparation of the silver nanoparticles was explained and characterized in our previous work (12).

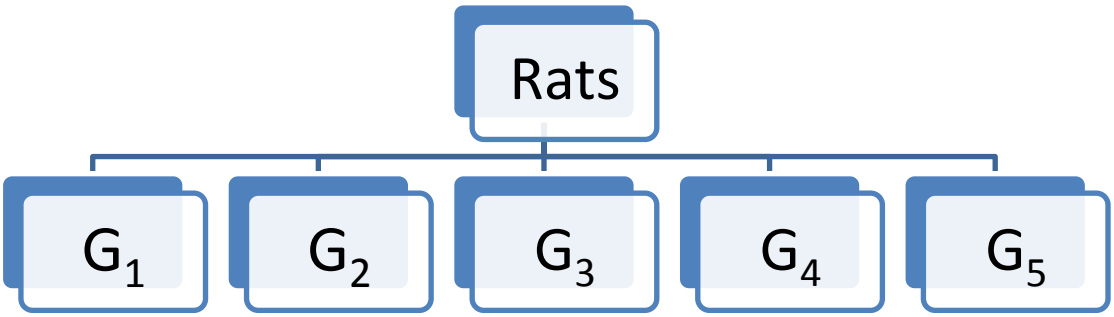
2-Determination of the Active Time and Active Dose:

The best active dose and active time of silver nanoparticles of Peel Extract of Raphanus sativus L was determined by giving a single intraperitoneal injection dose in different concentrations(50, 100, 150 ppm) and examined the effect of every dose at (0,2,4,6 and 24)hr. Designed this experiment to contain 24 rats,divided into 4 groups, each group 6 rats.

G_1 : control group (treated with normal saline), G_2: (intraperitoneal injection a single dose of 50ppm of sliver Nanoparticles extract), G_3: (intraperitoneal injection of 100ppm of sliver Nanoparticles extract)G_4: (intraperitoneal injection of 150ppm of sliver Nanoparticles extract) . Then the levels, glucose determination at (0,2,4,6,24) hr. At all the groups.

3- Experimental Design to Study the Hypoglycemic Effect

Adult male Wistar rats, weighing (200-350 g) were used for the test. Thirty rats were fed exclusively on fodder and water to drink and they received no other medication at the time outside of the extract silver nanoparticles. All rats subjected to the glucose level test before starting the experiments to conform rats without diabetic disease. They were randomly divided into five groups of 6 rats treated as follows**.**



Group 1: the control group(normal saline) . Group 2: considered as a diabetic group and received 150 mg/kg b.w of alloxan (i.p) as a single dose intraperitoneal injection .

Group3 : The overnight fasted rats were made diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate (Sigma Aldrich Germany; 150 mg/kg i.p.) in sterile saline. Then, treated with 100mg/kg b.w of extract silver nanoparticles for 60 days every day.

Group 4: (preventive group) treated with 1 00mg/kg b.w of extract silver nanoparticles for 60 days every day, Then as a diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate (Sigma Aldrich Germany; 150 mg/kg i.p.) in sterile saline.

G5: treated with 100mg/kg b.w of extract silver nanoparticles for 60 days every day by a single intraperitoneal injection of freshly prepared.

Determination of Biochemical Parameters

To assay blood glucose level, Aldolase A, glucose-6-phosphate dehydrogenase and Total antioxidant capacity in rats, we collected blood of these animals from the marginal heart, and the blood was collected in dry test tubes and anticoagulant (EDTA for glucose) and then centrifuged at 3000 rpm for 5 minutes, the serum was separated and stored for the determination of these parameters

1-Determination of Blood Glucose Concentration (mg/dl)

The blood glucose concentration was measured by the enzymatic method with GOD-PAP reagent the blood was determined after 60 days of administration of aqueous extract9(13).

The principle of the determination of glucose is based on the reaction

$$\textbf{Glucose} + 1/2\ O_2 + 2H_2O \rightarrow H_2O_2 + \textbf{Gluconic acid}$$

2-Determination of Aldolase A

Rat Aldoa(Aldolase A. Fructose Biophosphate) ELISA Kit .Catalog No. E-EL-R1174.96T.

3-Determination of Glucose-6-phosphate dehydrogenase Activity (IU/L) :

The principle of this method is based on Beutler method is as follows:

$$\text{G-6-P} + NADP^+ \xrightarrow{G-6-PDH} \text{6-PGluconate} + NADPH + H^+$$

The rate of NADPH concentration increasing in measured at 340nm is proportional to the G-6PDH activity in the specimen (14).

4-Determination of Blood Lactate Dehydrogenase activity

The measurement of Lactate Dehydrogenase- ELISA Kit (Biosystems (Spain)(15).

5-Determination of Total Antioxidant capacity

The measurement of total antioxidants based on spectrophotometric method within the kit supplied by Elabseiences Company(16) .

Statistical analysis

The results obtained were expressed as mean ± SD. The statistical comparison among the groups were performed with one way ANOVA and DMRT using statistical package (SPSS 24) at $p<0.05$.

Results and Discussion

1- The Active Time and Active Dose

The blood was collected from the rats of the groups, at different times 0,2,4,6 and 24 hours. The results in the Table (1) showed that two hours is the best time and the 100 ppm was the best dose of the silver nanoparticles extracts. The blood glucose level of rats was increased after two hours at 4, 6, and 24 hr. respectively.

The decreasing in blood glucose levels dependent with the increasing of dose of the AgNPs,but the dose 150 ppm causes a several death in rats so we excluded this dose and rely on the dose 100 ppm as the best dose

Table (1):The hypoglycemic effect of Silver Nanoparticles Using Peel Extract of *Raphanus sativus* L . In different times (0, 2, 4, 6 and 24) hours

Groups(Rats)	Glucose level mg/dl Time (hr.) 0	2	4	6	24
G_1(control)					
R_1	102	88	98	110	119
R_2	98	86	97	107	121
R_3	99	84	99	112	123
R_4	100	85	96	106	120
R_5	104	89	95	103	118
R6	105	91	94	101	117
G_2 (50 ppm)					
R_1	94	73	84	98	113
R_2	91	74	82	95	115
R_3	95	77	86	97	114
R_4	90	71	92	96	111
R_5	95	76	93	95	112
R_6	96	78	96	92	113
G_3(100ppm)					
R_1	101	83	96	100	102
R_2	98	78	93	98	105
R_3	103	81	95	99	108
R_4	97	82	97	101	101
R_5	99	87	96	102	103
R6	101	89	98	104	102
G_4(150 ppm)					
R_1	97	77	81	94	105
R_2	95	75	80	92	107
R_3	98	79	82	92	111
R_4	94	76	84	95	113
R_5	99	78	87	96	112
R6	101	80	89	97	114

2. Hypoglycemic effect of Silver Nanoparticles of Peel *Raphanus sativus* Extract:

The results in the Table (2) and Table (3) showed the ability of the AgNP of of *Raphuns sativus* extract in reducing of glucose levels whether they were fasting or randomly and this results give a perfect indicator of the hypoglycemic effect of

peel *Raphuns sativus*.L that related to the active component of the plant, which have been found to induce secretion or possess an insulin like effect (17) and can effect on pancreatic β-cells leading to their proliferation and secretion of more insulin. The active component in the AgNP of aqueous extract of *Raphancs sativus* may be responsible for increasing insulin release. Also, will promote induction of hepatic glucokinase, and the high in this enzyme activity cause directly demonstrates increased insulin secretion from β-cell (18)

Table: (2) Glucose Levels (mg/dl) Fasting for Rats Groups Compared with Control Group

Groups*	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	86.92 ±4.90a	2.00	81.78	92.06
G2	6	184.62±6.05b	2.47	178.27	190.98
G3	6	162.86±6.36c	2.60	156.19	169.55
G4	6	141.33±4.47d	1.82	136.64	146.03
G5	6	63.85±3.62e	1.48	60.04	67.65

***G_1 significant with G_2,G_3,G_4 ,G_5 at ($P \leq 0.05$) , G_2 significant with G_1,G_3,G_4 ,G_5 at ($P \leq 0.05$) ,G_3 significant with G_1,G_2,G_4 ,G_5 at ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_3 ,G_5 at ($P \leq 0.05$) , G_5 significant withG_1, G_2,G_3,G_4 .**

Table: (3) Random Glucose Levels (mg/dl) for Rats Groups Compared with Control

Groups*	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	119.63±1.73a	1.70	117.81	121.45
G2	6	289.35±23.90b	9.76	264.26	314.44
G3	6	181.85±4.82c	1.97	176.78	186.90
G4	6	154.76±4.11d	1.68	150.45	159.08
G5	6	108.039±5.18ae	2.11	102.60	113.48

G_1 significant with G_2,G_3,G_4 at ($P \leq 0.05$), no significantly different with G_5 ($P \leq 0.05$) , G_2 significant with G_1,G_3,G_4 ,G_5 at ($P \leq 0.05$) ,G_3 significant with G_1,G_2,G_4 ,G_5 at ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_3 ,G_5 at ($P \leq 0.05$) , G_5 significant with G_2,G_3,G_4 at ($P \leq 0.05$), no significantly with G_1 ($P \leq 0.05$).

3- Aldolase A Activity in diabetic rats

The activities of glycolytic enzymes like Aldolase A in the liver and kidney of control and experimental rats were clarified in Table (4). Intraperitoneal injection of Silver Nanoparticles of Raphanus sativus extract resulted in increased activity of Aldolase A. Treatment of diabetes rats groups also brought the activities near normal as in normal group

Table (4): Aldolase A (pg/ml) for Rats Groups Treated with Aqueous Extract of Silver Nanoparticles compared with Control Group

Groups*	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	63.44±5.72a	2.34	57.43	69.45
G2	6	40.15±2.89b	1.183	37.10	43.19
G3	6	44.22±2.69bc	1.10	41.39	47.06
G4	6	49.12±1.98cd	0.81	47.0402	51.2098
G5	6	52.32±2.68de	1.10	49.5072	55.1328

***G_1 significant with G_2,G_3,G_4 ,G_5 at ($P \leq 0.05$) , G_2 significant with G_1,G_4 ,G_5 at ($P \leq 0.05$) ,no significantly with G_3 ($P \leq 0.05$)) ,G_3 significant with G_1,G_4 ,G_5 at ($P \leq 0.05$) but not significantly with G_2 ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_5 at ($P \leq 0.05$), no significantly with G_3 ($P \leq 0.05$) , G_5 significant with G_1,G_2,G_3 at ($P \leq 0.05$), no significantly with G_4 ($P \leq 0.05$).**

The activity of aldolase in the presence of phosphate alone tends to be higher than in the presence of fructose 6-phosphate mono hydrogenase in the presence of both phosphate and fructose 6-phosphate significantly enhanced the activity of aldolase to a greater extent than that of phosphofructokinase. In different cases investigated in vivo, the activity of aldolase was similar in size to that of hexokinase, and somewhat less than phosphofructokinase. This result and Chan- gess in the activity of Aldolase on Famine and Diabetes suggested that Aldolase may be involved in controlling the decomposition of adipose tissue. To substantiate this assumption, the concentration of fatty lipid tissue, phosphofructokinase and aldolase activity in vivo was measured in different metabolic conditions (19,20) .

4- Glucose-6-phosphate dehydrogenase Activity in diabetic rats

Diabetes is a common and complicated disease. Studies imply glucose in the blood and its oxidation derivatives have a major role in the satisfactory process of blood.

Glucose 6 Phosphatedehydrogenase (G6PD), was an antioxidant enzyme and important in preventing its complications(21).

Increased glucose uptake of ROS in several cell layers with diabetes has been shown due to the combination of high ROS production along with reduced antioxidant function (22). Several laboratories have shown that pancreatic cells are very susceptible to oxidative damage, which are attributed to low expression levels of antioxidant enzymes. Thus, cells are likely to be at greater risk of oxidative-mediated cellular injury and death compared to alternative types of cell types 30. Table (5) illustrates G6PD for rat groups.

Table (5): G-6-PDH Activity (IU/L) for Rats Groups Treated with Aqueous Extract of Silver Nanoparticles Compared with Control Group

Groups*	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	119.63±1.73a	0.71	117.81	121.45
G2	6	289.36±23.90b	9.76	264.27	314.44
G3	6	181.85±4.82c	1.97	176.79	186.90
G4	6	154.77±4.11d	1.68	150.45	159.08
G5	6	108.04±5.18ae	2.11	102.60	113.47

***G_1 significant with G_2,G_3,G_4 at ($P \leq 0.05$), no significantly with G_5 ($P \leq 0.05$), G_2 significant with G_1,G_3,G_4 ,G_5 at ($P \leq 0.05$) ,G_3 significant with G_1,G_2,G_4 ,G_5 at ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_3 ,G_5 at ($P \leq 0.05$) , G_5 significant with G_2,G_3,G_4 at ($P \leq 0.05$), no significantly different with G_1 ($P \leq 0.05$).**

The results in the above table showed that the diabetic group has a high value, from the 6-phosphate glucose metabolized (G6PD) activity is the main source of intracellular reflux, NADPH, which is required by many enzymes, including the enzymes of the antioxidant pathway, G6PD activity in endothelial cells and kidneys decreases, leading to reduced cell survival. Pancreatic cells are highly sensitive to ROS increase (23).

Glycogenesis and /or gluconeogenesis increase the blood sugar level can be derived from diabetes in diet (24). In general, increased hepatic glucose production as well as a low level of hepatic glycogen synthesis and glycolysis are the major symptoms of type 2 diabetes that leads to hyperglycemia (25). These results revealed a massive depletion of hepatic glycogen contents. Our results are consistent with the results of Lavoi and van de Werf, 1991 (26) and Ahmed et al.

2010 (27) who found that a high level of glucose-6-phosphate activity and decreased hepatic glycogen content in diabetic mice.

5- Lactate Dehydrogenase Activity in diabetic rats

In the present study, Table (6) showed a significant increase of serum LDH level of group 2 comparison to the control group and other groups that showing the occurrence of hyperglycemia.

Table(6): Lactate Dehydrogenase Activity U/L for rats groups treated with aqueous extract of Silver Nanoparticles Using Peel Extract of *Raphanus sativus* L. with control group

Groups*	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	250.56±8.05a	3.29	242.10	259.01
G2	6	494.65±9.57b	3.90	484.60	504.70
G3	6	299.63±9.99c	4.08	289.13	310.11
G4	6	217.94±9.76d	3.17	209.80	226.09
G5	6	151.84±8.38e	3.42	143.05	160.64

G_1 significant with G_2,G_3,G_4 ,G_5 at ($P \leq 0.05$) , G_2 significant with G_1,G_3,G_4 ,G_5 at ($P \leq 0.05$) ,G_3 significant with G_1,G_2,G_4 ,G_5 at ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_3 ,G_5 at ($P \leq 0.05$) , G_5significant withG_1,G_2,G_3,G_4 at ($P \leq 0.05$) .

Increased activity of LDH indicates that diabetes may cause liver weakness. Support for our conclusion was found by through liver was necrotized in the diabetic group. Therefore, the increased activity of the LDH may be due mainly to the leakage of these enzymes from the liver cytosol in the bloodstream, which gives an indication of the liver effect of alloxan (28).

LDH plays an important role in carbohydrate metabolism and catalyses the inter-conversion of lactate and pyruvate. In the present study the brain LDH activity was significantly higher in diabetic rats. These results were similar to those of previous study that also demonstrated higher LDH activity in diabetic tissues. Lactate dehydrogenase (LDH) is a key of anaerobic glycolysis and catalyses the reversible oxidation of lactate to pyruvate in the terminal step of glycolysis. The reaction catalyzed by lactate dehydrogenase interlinks anaerobic and aerobic oxidation. Excessive accumulation of pyruvate may result in higher LDH activity during diabetic condition. Excessive pyruvate is converted into lactate for which LDH is

needed and, therefore, the activity of LDH may be increased due to less insulin availability in diabetes(29,30).

6- Total Antioxidant capacity (TOAC) :

Antioxidants inhibit interaction through free radicals with biomolecules and nutritional values and physiological properties of nutrients can be reminded (31). Antioxidant defense mechanisms include enzymatic and non-enzymatic strategies. The common non-enzymatic antioxidants contain vitamins A, E, C, α-lipoic acid, glutathione, mixed carotenoids, coenzyme Q10 (CoQ10), and many bioflavonoids and antioxidant minerals (copper, manganese, selenium and zinc), and auxiliary factors such as uric acid, folic acid, albumin, , B2, B6, and B12 (32) .

In Table (7), the results showed high levels of total oxidation in G3 and G4 when compared with the diabetic and control group .

Table(7) Total antioxidant Levels U/ml for rats groups treated with aqueous extract of *Sliver nanoparticlas* compared with control group

*Groups	N	Mean ±SD	S.E	95% Confidence	
				Lower	Upper
G1	6	8.30±0.44a	0.18	7.84	8.78
G2	6	6.14±0.16b	0.07	5.97	6.31
G3	6	7.01±0.25c	0.10	6.74	7.28
G4	6	7.53±0.13d	0.05	7.40	7.67
G5	6	7.93±0.10e	0.04	7.82	8.04

G_1 significant with G_2,G_3,G_4 ,G_5 at ($P \leq 0.05$) , G_2 significant with G_1,G_3,G_4 ,G_5 at ($P \leq 0.05$) ,G_3 significant with G_1,G_2,G_4 ,G_5 at ($P \leq 0.05$) , G_4 significant with G_1,G_2,G_3 ,G_5 at ($P \leq 0.05$) , G_5 significant withG_1,G_2,G_3,G_4 at ($P \leq 0.05$).

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