Hypoglycemic effect by assay some glucoregulatory enzymes and hematological parameters using silver nanoparticles of peel Raphanus sativus L aqueas extract in male Rats.
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Abstract:-
Hypoglycemic effect was investigated some glucoregulatory enzymes (Serum insulin level, superoxide dismutase (SOD), catalase activity) and hematological parameters in male rats divided into five groups six rats for each group:
G1 : control group (treated with normal saline), G2: diabetic groups induced by alloxan in a single dose (150 mg/kg b.w), G3: Diabetic groups (aloxan 150 mg/kg b.w intraperitoneal injection for 60 days + 100ppm of Nanoparticles extract), G4: preventive group(intraperitoneal injection for 60 days of 100ppm of Nanoparticles + alloxan in a single dose (150mg/kg b.w), G5:(Control treated with 100 ppm of nanoparticles for 60 days).

The results shown a significant increase in insulin level, red blood cells and hemoglobin content at (p<0.05) for the treated group(G3) and the preventive group(G4) when it was compared with diabetic group while decreased in the levels at (p<0.05) of catalase activity and white blood cells for the treated group(G3) and the preventive group(G4) when compared with diabetic group.

Keywords:
Raphanus sativus L, Silver nanoparticles AgNPs, Alloxan, Diabetes Mellitus, Hyperglycemia, insulin, red blood cell, white blood cell, hemoglobin, catalase, superoxide dismutase (SOD).

Introduction:-
Nanotechnology study’s the control and manipulation of materials at the nanoscales, typically having dimensions less than 100nm (Kulkarni and Muddapur, 2014). Nano word means the dwarf in ancient Greek (Mondal., et al., 2011).

Green synthesis of silver nano-particles using microorganisms including bacteria, fungi and plants because of their antioxidant properties capable of reducing metal compounds in their respective nanoparticle, Plant extracts produce best capping material for the stabilization of silver nanoparticles (Ahmed et al., 2015).
Medical plant have phytochemicals compound which are non-nutrient in nature but have good preventive action versus certain illness (Chede, 2012 and Ali et al., 2014).

Radish (Raphanus sativus L.) is a widely recognized medicinal root vegetable crop that was first domesticated in Europe (Lugasi et al. 2005). Radish is an ideal target for improving photosynthetic productivity because genome information for this crop is currently available (Kitashiba et al. 2014a).

The International Diabetic Federation (IDF), reported that over 246 million people globally suffer from diabetes which is expected to rise to 380 million by the year 2026 (IDF, 2017). Diabetes is a complex, chronic disease requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control (American Diabetes Association 2018).

Alloxan has a selective action on the β-cells of the islets of Langerhans the intraperitoneal route of alloxan is the most widely used method of induction of diabetes in lab animals because of its structural similarity to glucose, the structural integrity of the cytoskeleton, lysosomes, DNA and mitochondria would be lost, and the β-cells disintegrate resulting in a lack of insulin production when alloxan is taken up by the β-cells (Roja Rani et al., 2017).

Classification of diabetes is based on the etiology of the disease (Bilous and Donnelly., 2010). Etiologic classification of syndromes of glycaemia. Diabetes can be classified and modified as the following general categories according to (ADA, 2017).

Oxidative stress occurs in a biological system when there is over production of ROS and deficiency in enzymatic or non-enzymatic antioxidant (Rahman., et al., 2012).

The liquid component of blood is called plasma, a mixture of water, sugar, fat, protein, and salts. The main job of the plasma is to transport blood cells throughout the body along with nutrients, waste products, antibodies, clotting factors, chemical messengers such as hormones, and proteins that help maintain the body's fluid balance (American Society of Hematology, 2018).

Antioxidants are an important parts of the defense system in human body they help to cope with oxidative stress which is caused by reactive oxygen species. Antioxidant acts in stabilizing or deactivating free radicals, usually before attack of the target in biological cells (Numeset al., 2012).
Superoxide dismutase (SOD) readily converts highly reactive superoxide radical to a less reactive hydrogen peroxide (H$_2$O$_2$) in order to maintain an optimal cellular function (Speisky et al., 2009). Catalase (CAT) acts by breaking down formed (H$_2$O$_2$) in cells to molecular oxygen and water (Winter bourn, 2014).

Materials and methods:
1-preparation of the silver nanoparticles

The preparation of the silver nanoparticles was explained and characterized as in (Fadel Q . J and Al-Mashhedy LAM, 2017).

2-Laboratory Animals.

Adult male white albino rats with body weight of (200-350) gram, age 2-3 months were obtained from ministry of health national central for drug control and research (NCDCR). The rats housed under controlled animal conditions of temperature (25±3 C˚), animals were suitable for two weeks and they were maintained on a regular feed (control diet). Its contain (Crude protein 10%, ground soybean 20%, wheat flour 35%, corn 35%, mineral & vitamins 1 gm/Kg). Total energy was 13.6 KJ/Kg protein, All animal experiments were tested the level glucose previously.

3-Induction of hyperglycemia:

The rats were injected intravenous with alloxan monohydrate [(2,4,5,6) tetraoxyhexa hydro pyrimidine] to induce hyperglycemia after 12 hours fasting. The compound was freshly prepared (100mg/kg) dissolved in 2 ml of normal saline and given as single dose.

The rats have been taken 5% glucose with tap water for the first day only after dose of alloxan. Then left to relief and to eat enough after 3 days later the rats had hyperglycemia which indicated the blood glucose had more than 200 mg/dL and fatigue signs such as polyuria and polydipsia appeared.

4- Experimental Design:
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.
Group 3: 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 sal. Then, treated with 100mg/kg b.w of extract sliver nanoparticles for 60 days every day.
Group 4: treated with 100mg/kg b.w of extract sliver 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 Sal.
Group 5: treated with 100mg/kg b.w of extract sliver nanoparticles for 60 days every day by a single intraperitoneal injection of freshly prepare.
5-Determination of Insulin
used ELISA Kit(Cal biotech. USA).
6-Determination of Superoxide Dismutase (SOD) Activity

( Cu – Zn )SOD activity is determined by (Luc Magnani et al ,2000 and Marklund , S et al., 1974).
7-Determination of Catalase Activity

Catalase activity was determined according to the method described previously by( Sinha AK.,1972 and Mahmoud H., 2016).
8-Complete blood count (CBC) blood picture:-
CBC were measured by use full automated blood analyzer (mythic, France.
9- Statistical Analysis

Statistical analysis was performed using SPSS-24 (Statistical Packages for Social Sciences- version 24). Data were analyzed using one-way analysis of G variance (ANOVA one way ). Least significant differences (LSD) post hoc test was performed (multiple comparisons), to assess significant difference among means( P < 0.05) that was considered statistically significant.
Results and Discussion

1- Serum Insulin Level

In the table (1) results showed significant decrease (P<0.05) in fasting serum insulin in diabetic rats compared to normal control rats. This might be due to the partial destruction of pancreatic β-cells by the direct effect of alloxan, because alloxan is well known to cause selective hydropic degeneration, degranulation, necrosis of the pancreatic β-cells, and fibrosis of the islets in a dose depending pattern (Zhang et al., 2003; Singh and Gupta, 2007).

| Groups | Mean ±SD (µIU/ml) | Lower | Upper |
|--------|------------------|-------|-------|
| N      | 95% Confidence   |       |       |
| G1     | 11.85±2.91       | 9.79  | 14.90 |
| G2     | 3.09±0.27        | 2.80  | 3.38  |
| G3     | 4.43±0.38        | 4.03  | 4.83  |
| G4     | 8.72±0.43        | 6.87  | 9.17  |
| G5     | 10.56±1.44       | 5.78  | 9.08  |

N*= Number of rats , LSD = 1.764 Values represent mean ± SD Mean± Standard Deviation Mean differences is significant p ≤ 0.05 level

In treatment groups, insulin levels returned near normal value as a result, this result reflects the hypoglycemic effect of the mixture suggesting that the hypoglycemic effect might be mediated through potentiation of pancreatic secretion of insulin from β-cell of islets. Another possible hypoglycemic mechanism of AgNP₅ extracts might increase the sensitivity of tissue to available insulin. This effect of mixture might be because containing natural antioxidant compound which reduced ROS formation in β-cell induced by alloxan and enhance
the defense antioxidant mechanism against ROS production in diabetes type 2 (Maraia., et al 2017).

2-Oxidative Stress in Diabetes Mellitus

Oxidative stress played an important role in the development of vascular complications in diabetes particularly type 2 diabetes (Pham-Huy, 2008). ROS level elevation in diabetes might be due to decrease in destruction or/and increase in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD). The variation in the levels of these enzymes made the tissues susceptible to oxidative stress leading to the development of diabetic complications (Lipinski,2001).

2-1- Superoxide Dismutase (SOD)

In the table (2) results showed significant decrease (P<0.05) of the SOD activity in diabetic rats group compared to normal control rats group. Superoxide dismutase provides first line defense against ROS mediated cell injury by catalyzing the proportion of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide that superoxide was dismasted to other compounds that are less toxic by SOD (Tiwari et al., 2013).

Table (2): level of SOD activity ( U/ml ) for rats groups treated with aqueous extract of silver nanoparticles using peel extract of Raphanus sativus L. with control group

| Groups* | Mean ±SD | 95% Confidence |
|---------|---------|----------------|
|         | Lower  | Upper         |
| N*=6    |        |               |
| G1      | 3.41±0.15 | 3.25 | 3.57 |
| G2      | 1.45±0.16 | 1.28 | 1.62 |
| G3      | 2.11±0.06 | 2.03 | 2.18 |
| G4      | 2.51±0.07 | 2.43 | 2.60 |
| G5      | 3.76±0.11 | 2.93 | 3.98 |

N*= Number of rats, LSD = 0.144 Values represent mean ± SD: Mean± Standard Deviation Mean differences is significant p ≤ 0.05 level
The reduced activity of SOD in these tissues could be as a result of an increased demand for this enzyme in deactivating the high influx of reactive oxygen species generated by induction of diabetes. It could also as a result of insufficiency of the enzyme or failure of the antioxidant system to overcome the influx of reactive oxygen species. Furthermore, it could be because of the creation of oxidative atmosphere in tissue by impairment in the functioning of endogenous antioxidant like SOD due to alloxan-induced diabetes (Kamesh and Sumathi, 2012).

2.2-Catalase Activity

Results in table (3) showed significant increase (P<0.05) in level of CAT activity in diabetic rats group compared to normal control rats group and other groups.

Table (3): Catalase activity( kU) for rats groups treated with aqueous extract of silver nanoparticles using peel extract of *Raphanussativus* L. with control group

| Groups | Mean ±SD | 95% Confidence Interval for Mean |
|--------|----------|--------------------------------|
|        | Lower    | Upper                         |
| N=6    |          |                               |
| G1     | 17.50±0.87 | 16.59     | 18.42     |
| G2     | 26.69±0.70 | 25.95     | 27.43     |
| G3     | 21.43±0.63 | 20.76     | 22.10     |
| G4     | 19.83±0.28 | 19.53     | 20.12     |
| G5     | 18.86±0.68 | 18.14     | 19.57     |

N= Number of rats , LSD = 0.790 Values represent mean ± SD: Mean± Standard Deviation. Mean differences is significant p ≤ 0.05 level

The level of CAT activity increase after treatment by AgNP₅ in the G₃,G₄, G₅ groups when it is compared with G₁ control group because AgNPs could decrease oxidative stress this led to the increasing of the antioxidant in the treatment groups( Kakkar R et al ,1995).

3-White , red blood and hemoglobin cells Count

WBC count of diabetic rats showed significant increasing than the control its value (11.87 ± 0.69 x 10³/mm³) (P < 0.05) while control value (8.01 ± 0.64 x 10³/mm³). Also group 3 and 4 differs significantly than diabetic rats than diabetic rats there value (9.85 ± 0.47 and 8.42 ± 0.59 x 10⁶/mm³ ) respectively in (Table 4).

RBC count of diabetic control rats(6.17 ± 0.30 x 10⁶/mm³) showed a significant decrease (P < 0.05) as compared to normal control rats (7.75 ± 0.38 x 10⁶/mm³) in (Table 5).
The recorded values of diabetic rats showed significant decrease in blood hemoglobin content ($P < 0.05$) (10.71 ± 0.97 g/dl) as it was compared with the normal control rats (14.95 ± 0.34 g/dl). Treatment with silver nanoparticles extract produced a highly significant increase of the blood hemoglobin content of diabetic rats; the values being 14.48 ± 0.62 and 14.33 ± 0.49 g/dl, respectively in (Table 6).

**Table (4):** White blood cells (cell×10³/mm³) for rats groups treated with aqueous extract of silver nanoparticles using peel extract of *Raphanussativus* L. with control group

| Groups* | Mean ±SD (cell×10³/mm³) | 95% Confidence | Lower | Upper |
|---------|--------------------------|----------------|-------|-------|
| G1      | 8.01±0.64                | 7.53           | 10.71 |
| G2      | 11.87±0.69               | 10.14          | 13.61 |
| G3      | 9.85±0.47                | 8.31           | 10.50 |
| G4      | 8.42±0.59                | 7.10           | 9.04  |
| G5      | 8.12±0.21                | 6.52           | 9.15  |

*N* = Number of rats, LSD = 0.657 Values represent mean ± SD: Mean± Standard Deviation Mean differences is significant $p \leq 0.05$ level

**Table (5):** Red blood cells (cell×10⁶/mm³) for rats groups treated with aqueous extract of silver nanoparticles using peel extract of *Raphanussativus* L. with control group

| Groups* | Mean ±SD (cell×10⁶/mm³) | 95% Confidence | Lower | Upper |
|---------|--------------------------|----------------|-------|-------|
| G1      | 7.75±0.38                | 6.35           | 9.16  |
| G2      | 6.17±0.30                | 5.06           | 7.13  |
| G3      | 6.32±0.29                | 5.39           | 7.00  |
| G4      | 7.17±0.28                | 6.04           | 8.34  |
| G5      | 7.41±0.11                | 6.10           | 8.78  |

*N* = Number of rats, LSD = 0.343 Values represent mean ± SD: Mean± Standard Deviation Mean differences is significant $p \leq 0.05$ level
Table (6): Hb level blood (g/dl) for rats groups treated with aqueous extract of silver nanoparticles using peel extract of \textit{Raphanussativus} L. with control group

| Groups* | Mean ±SD (g/dl) | 95% Confidence |
|---------|-----------------|----------------|
|         | N               | Lower | Upper |
| G1      | 14.95±0.34      | 12.78 | 16.10 |
| G2      | 10.71±0.97      | 8.68  | 11.73 |
| G3      | 11.27±0.70      | 9.42  | 13.91 |
| G4      | 14.10±0.68      | 10.95 | 14.82 |
| G5      | 14.25±0.60      | 11.41 | 15.01 |

\( N^* = \) Number of rats , \( \text{LSD} = 0.826 \) Values represent mean ± SD: Mean± Standard Deviation Mean differences is significant \( p \leq 0.05 \) level

A reduction in the number of WBC could also be as a result of diabetes induced stress which breaks down the rat defensive mechanism (Heistad ., et al 2006) . Diabetic rats treated with all doses of the extracts showed improvement in WBC count. Mechanism of experiment could probably be due to the fact that the extract contains some constituents that stimulate and/or promote the production of WBC and hence offer some form of protection to the rat immune system(Ampa., et al 2017 ; Maritim ., et al 2003).

Conclusions

The conclusions of this study indicate that:-

Silver nanoparticles extracts can be used as hypoglycemic agent against diabetes millets that induced by alloxan in rats.

References :-

[1] Ahmed, E. A. \textit{et al.} (2015) ‘Biosynthesis of silver nanoparticles by \textit{Spirulina platensis} and \textit{Nostoc sp’}, \textit{Glo. Adv. Res. J. Microbiol}, 4(4), pp. 36–49.
[2] Ali, S. \textit{et al.} (2014) ‘Comparative studies of various phyto nutrients in citrus fruits’, \textit{Pak. J. Chem}, 4(2), pp. 72–76.
[3] American Diabetes Association Standards of medical care in diabetes—2018. Diabetes Care. 2018;41(Suppl 1):S1–S2.
[4] Ampa, k. et al. (2017) Fasting Blood Glucose Levels and Hematological Values in Normal and Streptozotocin-Induced Diabetic Rats of Mimosa pudica L. Extracts. Pharmacogn J. 9(3): 315-322.

[5] Association, A.D. (2017) ‘Diagnosis and classification of diabetes mellitus’, Diabetes care. Am Diabetes Assoc, 37(Supplement 1), pp. S81–S90.

[6] Bilous, R. and Donnelly, R. (2010) Handbook of diabetes. John Wiley & Sons.

[7] Chede, P. S. (2012) ‘Phytochemical Analysis of Citrus sinensis Pulp’, International Journal of Pharmacognosy and Phytochemical Research, 4(4), pp. 221–223.

[8] Fadel QJ and Al-Mashhedy LAM, (2017). Biosynthesis of Silver Nanoparticles Using Peel Extract of Raphanus sativus L. biotechnology An Indian journal ; 13:1:1-10.

[9] Hadwan, M. H. (2016) ‘New method for assessment of serum catalase activity’, Indian Journal of Science and Technology, 9(4).

[10] International Diabetes Federation. IDF diabetes atlas. 8. Brussels: IDF; 2017.

[11] Kakkar, R. et al. (1995) ‘Lipid peroxidation and activity of antioxidant enzymes in diabetic rats’, Molecular and cellular biochemistry. Springer, 151(2), pp. 113–119.

[12] Kamesh, V. and Sumathi, T. (2012) ‘Antihypercholesterolemic effect of Bacopa monniera linn. on high cholesterol diet induced hypercholesterolemia in rats’, Asian Pacific Journal of Tropical Medicine. Elsevier, 5(12), pp. 949–955.

[13] Kitashiba, H. et al. (2014) ‘Draft sequences of the radish (Raphanus sativus L.) genome’, DNA research. Oxford University Press, 21(5), pp. 481–490.

[14] Kulkarni, N. and Muddapur, U. (2014) ‘Biosynthesis of metal nanoparticles: a review’, Journal of Nanotechnology. Hindawi, 2014.

[15] Lipinski, C.A., et al. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 46, 3-26.

[16] Luc Magnani, M. Gaydou, Jean Claude Hubaud(2000) . Spectrophotometric measurement of antioxidant properties of flavones and flavones against superoxide anoin , Anal. Chim. Acta 411 , 1 – 2 , 1 : pp . 209 – 16.

[17] Lugasi, A. et al. (2005) ‘Antioxidant effect of squeezed juice
from black radish (Raphanus sativus L. var niger) in alimentary hyperlipidaemia in rats', Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. Wiley Online Library, 19(7), pp. 587–591.

[18] Maria, E., et al. (2017). ‘Antioxidant effects of vitamins in type 2 diabetes: a meta-analysis of randomized controlled trials’ Diabetol Metab Syndr. 2018; 10: 18.

[19] Maritim, A. C., Sanders, aRA and Watkins lIi, J. B. (2003) ‘Diabetes, oxidative stress, and antioxidants: a review’, Journal of biochemical and molecular toxicology. Wiley Online Library, 17(1), pp. 24–38.

[20] Marklund , S. and , G(1974). Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase . Eur. J. Biochem ; 47 : 469 – 74.

[21] Mondal, A. K. et al. (2011) ‘Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex used as an Important Taxonomic Tool for Phylogenetic Interrelationship Advances in Biresearch Vol. 2’, Synthesis, 31, p. 33.

[22] Nunes, X. P. et al. (2012) ‘Biological oxidations and antioxidant activity of natural products’, in Phytochemicals as nutraceuticals-Global Approaches to Their Role in Nutrition and Health. InTech.

[23] Pham-Huy, L. A., He, H. and Pham-Huy, C. (2008) ‘Free radicals, antioxidants in disease and health’, International journal of biomedical science: IJBS. Master Publishing Group, 4(2), p. 89.

[24] Rahman, T. et al. (2012) ‘Oxidative stress and human health’, Advances in Bioscience and Biotechnology. Scientific Research Publishing, 3(07), p. 997.

[25] Roja, R. et al. (2017) ‘A histological study of alloxan-induced diabetes on experimental male Wistar rats’ National Journal of Physiology, Pharmacy and Pharmacology ,7 (12), pp.1329-1334.

[26] Singh, N. and Gupta, M. (2007) ‘Regeneration of β cells in islets of langerhans of pancreas of alloxan diabetic rats by acetone extract of Momordica charantia (Linn.)(bitter gourd) fruits’. CSIR.

[27] Sinha, A. K. (1972) ‘Colorimetric assay of catalase’, Analytical biochemistry. Elsevier, 47(2), pp. 389–394.

[28] Speisky, H. et al. (2009) ‘Generation of superoxide radicals by copper–glutathione complexes: redox-consequences associated with their interaction with reduced glutathione’, Bioorganic & medicinal chemistry. Elsevier, 17(5), pp. 1803–1810.

[29] Tiwari, B. K. et al. (2013) ‘Markers of oxidative stress during
diabetes mellitus’, *Journal of biomarkers*. Hindawi, 2013.

[30] Winterbourn, C. C. (2014) ‘The challenges of using fluorescent probes to detect and quantify specific reactive oxygen species in living cells’, *Biochimica et Biophysica Acta (BBA)-General Subjects*. Elsevier, 1840(2), pp. 730–738.

[31] Zhang, H.-N. *et al.* (2003) ‘In vitro and in vivo protective effect of Ganoderma lucidum polysaccharides on alloxan-induced pancreatic islets damage’, *Life sciences*. Elsevier, 73(18), pp. 2307–2319.