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**Scientific Report in Clinical Biochemistry**

# Evaluation of Its Antioxidant Activity in Induce type -2 Diabetic patients

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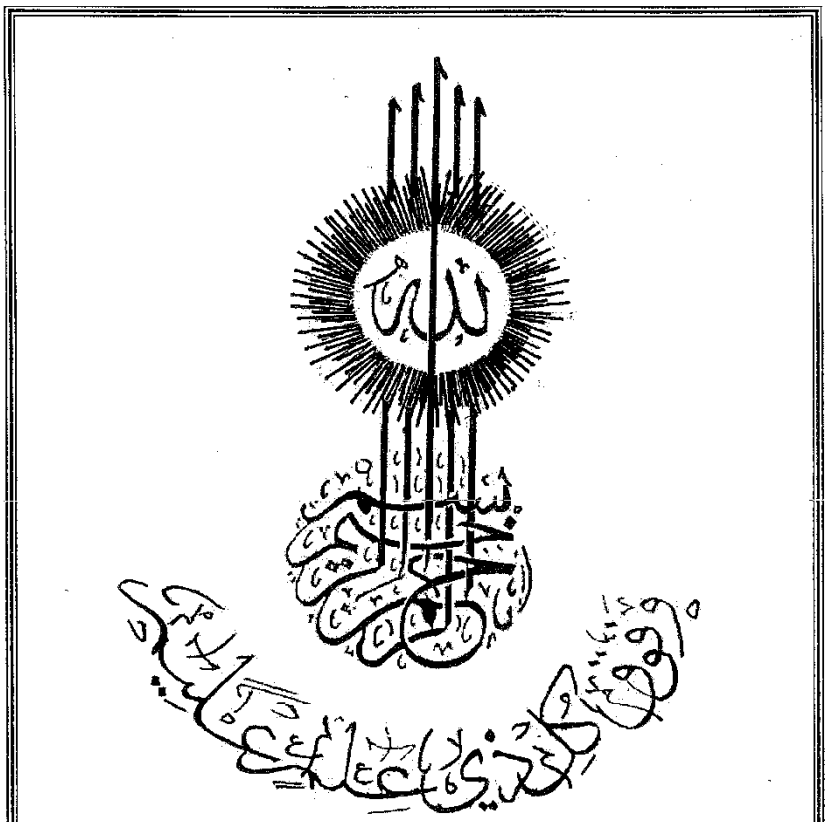

صدق الله العلي العظيم

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# الاهداء

إليك يا أمي يا من علمتني العطاء دون انتظار المقابل، يا من زرعتي في قلبي أسمى المعاني الأفاضل ..

إلى ذلك الصرح العظيم الذي علمني الخلق الكريم ، والدي صاحب الفضل الكبير ..

إليك يا أستاذي الكريم الذي علمتني أن تشجيع المعلم لتلميذه دافع قوي له على التقدم ..

إلى إخوتي وأخواتي سندي في حياتي . إلى كل من دعمني وشجعني في حياتي وأعطاني دفعة نحو الأمام ..

## Introduction

### Diabetes Mellitus

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, 2006). Diabetes is a complex, chronic disease requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control(American Diabetes Association 2017).

Hyperglycemia in DM has been associated with increased formation of ROS which are chemically reactive molecules containing oxygen that damage important tissues and organs in the body with the upregulation of inflammatory mediators (Anzar, 2013).

Diabetes is associated with the destruction of pancreatic β-cells which consequently leads to insulin deficiency resulting in insulin resistance (ADA,2010). Diabetes is characterized by chronic hyperglycemia, a very common metabolic abnormality among diabetic individuals inducing increased ROS generation which subsequently lead oxidative stress , Hyperglycemia is associated with failure of various tissues ; the liver, eyes, kidneys, blood vessels, nerves, heart, and damage to macromolecules (Dutta et al., 2015).

Hyperglycemia causes oxidative stress via several mechanisms leading to a rise in the generation of advanced glycated end products (AGEs), formation of superoxide radical, as well as increase in protein glycosylation, inflammatory mediators and glucose autoxidation .

Consequently, when overproduction of ROS occurs, this surpasses the antioxidant system’s capacity to counterbalance and eliminate these species, subsequently resulting in oxidative stress (Rahman et al., 2012 ).

The oxidative stress in the diabetic state is due to autoxidation of glucose level which usually leads to free radical generation and disruption of cellular homeostasis (Khan et al., 2015).

As of 2019, an estimated 463 million people had diabetes worldwide (8.8% of the adult population), with type 2 diabetes making up about 90% of the cases. Rates are similar in women and men. Trends suggest that rates will continue to rise. Diabetes at least doubles a person's risk of early death. In 2019, diabetes resulted in approximately 4.2 million

deaths. It is the 7th leading cause of death both globally and in the US. The global economic cost of diabetes related health expenditure in 2017 was estimated at US$727 billion. In the United States, diabetes cost nearly US$327 billion in 2017. Average medical expenditures among people with diabetes are about 2.3 times higher.

**Classification of Diabetes Miletus**

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).

**Type I -** is an autoimmune disease which destroys insulin producing pancreatic cells, whereby no insulin is secreted as a result of pancreatic β-cell deterioration and reliance on exogenous insulin for survival . About 10% of all diabetics are type 1 with dependence on insulin for survival with risk of ketoacidosis (ADA, 2010). Since this type is auto-immune, a preventive regime is yet to be known.

**Type II-** is a progressive disease typified by insufficient production of insulin or insulin resistance (Mohamed et al., 2016). About 90% of all diabetes incidences are type 2 and it is the second highest risk factor for developing Alzheimer's disease (Breteler, 2000 and CDCP., 2011). Oxidative stress has been implicated in the pathology of type 2 diabetes (Evans et al., 2002 ; Giacco and Brownlee, 2010).

**Gestational diabetes-** is most common in pregnant women. It is characterized by a rise in glucose level and insufficient insulin which reduces glucose level. A large number of individuals develop pre diabetes before a diabetic condition is diagnosed.

Prediabetes, the glucose level is consistently above normal and often progresses to type 2 diabetes. Gestational diabetes is characterized by a slight resistance to insulin (Metzger et al., 2007).

**Monogenic Diabetes -** has been newly diagnosed and which is characterized by a single gene mutation in the mitochondrial DNA or the autosomal dominant inheritance pattern. It is most common in young individuals ( Chan, 2016).

**Signs and symptoms of diabetes mellitus :-**

Frequent urination

Excessive thirst

Unexplained weight loss

Extreme hunger

Sudden vision changes

Tingling or numbness in the hands or feet

Feeling very tired much of the time

Very dry skin

Sores that are slow to heal

More infections than usual

Some people may experience only a few symptoms that are listed above. About 50 percent of people with type 2 diabetes don't experience any symptoms and don't know they have the disease

**Complications of diabetes mellitus :-**

heart disease

stroke

nerve damage, or neuropathy

foot problems

kidney disease, which can result in a person needing dialysis

eye disease or loss of vision

sexual problems in both men and women

Untreated diabetes can also lead to hyperosmolar hyperglycemic nonketotic syndrome (HHNS), which causes a severe and persistent increase in blood sugar levels.

**Risk factors for type 2 diabetes:-**

being 45 years of age or older

living a sedentary lifestyle

being overweight or obese

eating an unhealthful diet

having a family history of diabetes

having polycystic ovary syndrome (PCOS)

having a medical history of gestational diabetes, heart disease, or stroke

having pre diabetes being of African American, Alaska Native, Hispanic or Latino, American Indian, Asian American, Native Hawaiian, or Pacific Islander descent
American

**Reactive Oxygen Species and Oxidative Stress in Diabetes**

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). Oxygen is essential for metabolic

processes to take place. However, oxygen is the main source of free radicals. Many elements comprise molecules which have electrons in their outer orbital. Chemical bonds are formed between these elements dissociate during oxidation such as oxidative phosphorylation in mitochondria resulting in highly reactive species with unpaired electrons in their outermost orbital. Molecular oxygen is reduced by one electron during oxidation to form superoxide radical ($O_2^{\cdot-}$) a precursor for most ROS. Superoxide radical further reacts with an electron to form hydrogen peroxide ($H_2O_2$).

Hydrogen peroxide can be partially reduced to hydroxyl anion ($OH^-$) or fully reduced to water. ROS are either free radicals, e.g. hydroxyl radical ($^{\cdot}OH$), peroxyl radical, superoxide radical ($O_2^{\cdot-}$)ozone ($O_3$), or non-radical reactive compounds such as singlet oxygen (O2) and hydrogen peroxide ($H_2O_2$) .

Free radicals are atoms or molecules with lone pair of electrons and are capable of independent existence (Murdolo et al., 2013). Free radicals have been implicated in a number of disease conditions including diabetes (Murdolo et al., 2013).

Factors that promote reactive nitrogen species generation include normal metabolic processes such as aging, stress, poor diet, pollution, radiation, infection, disruption of the nitric oxide pathway and drugs as well as an inflammatory response (Zhang et al., 2002). These reactive species (hydroxyl, peroxyl, superoxide radical and hydrogen peroxide) initiate cumulative damage to proteins, lipids and nucleic acids and eventually lead to change in structure and function of organs and cellular components (Halliwell and Gutteridge, 2015).

**Glucose**

Hyperglycemia raise of glucose concentrations damages β-cell function and action, a phenomenon known as “glucotoxicity.” has always been linked with improvement in β-cell function. The key mechanisms accounting for the bad effect of high glucose levels is activation of oxidative stress, as a results of increased glucose oxidation in the mitochondria, mitochondrial dysfunction, and overproduction of reactive oxygen species (ROS) .

In contrast, over expression of antioxidant factors decreases the level of indicators of oxidative stress and rises the β-cell response to insulin (Marchetti, *et al.*,2013).

**Antioxidant Biomolecules**

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).

The role of free radical deactivation in pathology is known to be involved in many disease such as diabetes, atherosclerosis, aging, immune suppression and neuro degeneration (Sukandar et al., 2015).

**Enzymatic Antioxidants**

**Superoxide dismutase (SOD)** readily converts highly reactive superoxide radical to a less reactive hydrogen peroxide ($H_2O_2$) in order to maintain an optimal cellular function .

Catalase (CAT) acts by breaking down formed ($H_2O_2$) in cells to molecular oxygen and water (Winterbourn, 2014). Glutathione peroxidase (GPx) is a selenocysteine-containing enzyme and it functions in scavenging and removing hydrogen peroxide and lipid peroxide from cells. It does this in the presence of reduced GSH and NADPH. GPx reduces $H_2O_2$ and organic peroxide to water and alcohol thereby inhibiting the formation of free radicals (Rani and Yadav., 2015).

**High-density lipoprotein (HDL)**

is one of the five major groups of lipoproteins. Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells. They are typically composed of 80–100 proteins per particle (organized by one, two or three ApoA; more as the particles enlarge picking up and carrying more fat molecules) and transporting up to hundreds of fat molecules per particle.

lipid constituents of HDL particles include glycerophospholipids, cholesteryl esters (ChoE), sphingomyelins (SM), and triacylglycerols (TG). Lysophosphatidylcholines (lysoPC) are known to be associated with proatherogenic conditions

Normal range of HDL grouphealthy HDL levelage 19 or youngermore than 45mg/dl men age 20 or oldermore than 40mg/dl women age 20 or oldermore than 50mg/dl

**Non-enzymatic Antioxidants :-**

Exogenous antioxidant sources include vitamin C, E, A, carotenoids, phenols and glucosinolates which protect cells from free radicals generation and stabilizing radicals . Presently, there has been appreciable interest in various therapeutic remedies to the disease conditions with minimal or negligible side effects (Al-Snafi, 2015).

In recent times, medicinal plants including MO have attracted the attention of researchers for their therapeutic and phytochemical properties in treating and managing various diseases including DM (Sreelatha and Padma, 2009; Kumari, 2010; Wang et al., 2012; Jung, 2014)

**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).

Valko *et al*., (2006), demonstrated that the superoxide dismutase (SOD) is one of the most efficient intracellular enzymatic antioxidants. It is the antioxidant enzyme that catalyzes the dismutation of $O_2^{\circ-}$ to O2 and

to the less reactive species $H_2O_2$ (hydrogen peroxide). Diabetic control groups showed low level compared with patients group .

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 ).

**How obesity increases your risk for type 2 diabetes**

People who are obese have a high risk of developing type 2 diabetes, which is also known as insulin-resistant or adult-onset diabetes. This is a In obese .condition where your blood glucose level is persistently high persons, cells of fat tissues have to process more nutrients than they can manage. The stress in these cells triggers an inflammation that releases a protein known as cytokines. Cytokines then block the signals of insulin .receptors, thus gradually causing the cells to become resistant to insulin

Insulin allows your cells to use glucose (sugar) for energy. When you are resistant to insulin, your body is unable to convert the glucose into energy and you end up with a persistently high blood glucose level. Besides suppressing normal responses to insulin, the stress also triggers . .inflammation in cells that can lead to heart disease

According to health experts, such a rise would result in more than a million extra cases of type 2 diabetes, heart disease and cancer .

**LINKS BETWEEN OBESITY AND TYPE 2 DIABETES**

While the exact causes of diabetes are still not fully understood, it is known that factors up the risk of developing different types of diabetes .mellitus

For type 2 diabetes, this includes being overweight or obese (having a body mass index – BMI – of 30 or greater )

In fact, obesity is believed to account for 80-85% of the risk of developing type 2 diabetes, while recent research suggests that obese people are up to 80 times more likely to develop type 2 diabetes than .those with a BMI of less than 22 .

## HOW DOES OBESITY CAUSE TYPE 2 DIABETES

It is a well-known fact that if you are overweight or obese, you are at greater risk of developing type 2 diabetes, particularly if you have excess .weight around your tummy (abdomen)

## INFLAMMATORY RESPONSE

Studies suggest that abdominal fat causes fat cells to release 'pro-inflammatory' chemicals, which can make the body less sensitive to the insulin it produces by disrupting the function of insulin responsive cells and their ability to respond to insulin.

This is known as insulin resistance – the hallmark of type 2 diabetes

Having excess abdominal fat (i.e. a large waistline) is known as central or abdominal obesity, a particularly high-risk form of obesity**.**

**References:-**


Al-Snafi, A. E. (2015) 'Therapeutic properties of medicinal plants: a review of plants with cardiovascular effects (part 1)', *Int J of Pharmacology & Toxicology*, 5(3), pp. 163–176.

Ansari, N. A. and Dash, D. (2013) 'Amadori glycated proteins: role in production of autoantibodies in diabetes mellitus and effect of inhibitors on non-enzymatic glycation', *Aging and disease*. JKL International LLC, 4(1), p. 50.

Association, A. D. (2014) 'Diagnosis and classification of diabetes mellitus', *Diabetes care*. Am Diabetes Assoc, 37(Supplement 1), pp. S81–S90.

Association, A. D. (2017) 'Diagnosis and classification of diabetes mellitus', *Diabetes care*. Am Diabetes Assoc, 67(Supplement 1), pp. 650–657.

Atlas, D. (2006) 'International diabetes federation', *Press Release, Cape Town, South Africa*, 4.

Chen, L., Magliano, D. J. and Zimmet, P. Z. (2016) 'The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives', *Nature reviews endocrinology*. Nature Publishing Group, 8(4), p. 228.

Del Guerra, S. *et al.* (2015) 'Functional and molecular defects of pancreatic islets in human type 2 diabetes', *Diabetes*. Am Diabetes Assoc, 54(3), pp. 727–735.

Harries, A. D. *et al.* (2016) 'Addressing diabetes mellitus as part of the strategy for ending TB', *Transactions of the Royal Society of Tropical Medicine and Hygiene*. Royal Society of Tropical Medicine and Hygiene, 110(3), pp. 173–179.

Hasanein, P., Felehgari, Z. and Emamjomeh, A. (2016) 'Preventive effects of Salvia officinalis L. against learning and memory deficit induced by diabetes in rats: Possible hypoglycaemic and antioxidant mechanisms', *Neuroscience letters*. Elsevier, 622, pp. 72–77.

Kamesh, V. and Sumathi, T. (2014) 'Nephroprotective potential of Bacopa monniera on hypercholesterolemia induced nephropathy via the NO signaling pathway', *Pharmaceutical biology*. Taylor & Francis, 52(10), pp. 1327–1334.

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 .

Marchetti, P. *et al.* (2013) 'The pancreatic β cells in human type 2 diabetes', in *Diabetes*. Springer, pp. 288–309.

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.

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Mohamed, A. S., Soliman, A. M. and Marie, M. A. S. (2016) 'Mechanisms of echinochrome potency in modulating diabetic complications in liver', *Life sciences*. Elsevier, 151, pp. 41–49.

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.

Pitocco, D. *et al.* (2013) 'Oxidative stress in diabetes: implications for vascular and other complications', *International journal of molecular sciences*. Multidisciplinary Digital Publishing Institute, 14(11), pp. 21525–21550.

Rahman, T. *et al.* (2012) 'Oxidative stress and human health', *Advances in Bioscience and Biotechnology*. Scientific Research Publishing, 3(07), p. 997.

Rani, V. and Yadav, U. C. S. (2014) *Free radicals in human health and disease*. Springer.

Tiwari, B. K. *et al.* (2013) 'Markers of oxidative stress during diabetes mellitus', *Journal of biomarkers*. Hindawi, 2013.

Valko, M. *et al.* (2006) 'Free radicals, metals and antioxidants in oxidative stress-induced cancer', *Chemico-biological interactions*. Elsevier, 160(1), pp. 1–40.