Analytical Study on Activities of Enzymes and Antioxidants on Diabetic and Non Diabetic Cataract Patients

M. Noor Rezwana[1], Dr. Kamlesh Kumar[2]

[1]Research Scholar, OPJS University, Churu, Rajasthan
[2]Assistant Professor, OPJS University, Churu, Rajasthan

Abstract

Biochemical data shows that oxidative damage to lens proteins plays a role in the origin of senile cataract and the progression of diabetes-related pathologic alterations, including the development of cataracts. This species may cause damage to the pancreatic islets, which might explain why diabetics have trouble producing as much insulin. Multiple phytocompounds found in medicinal plants have been shown to limit this enzyme's activity. Interaction with reactive oxygen species may lead to lipid alteration (ROS) Cataracts are the most common cause of blindness and vision impairment across the globe. Cataracts are clouding of the lens of the eye, brought on by age or diabetes (DM) Patients with senile cataract and senile cataract with diabetes mellitus had their lens capsule fluid tested for Cu, Zn superoxide dismutase activity after cataract surgery.

Keywords: *Cataract, Diabetes Mellitus, Oxidative Stress, Superoxide Dismutase Enzyme.*

1. INTRODUCTION

Some diabetic problems may develop and worsen due to the oxidative stress brought on by diabetes mellitus. Cataracts are a degenerative complication of diabetes, and it has been hypothesized that free oxygen radicals play a role in their development. Antioxidants such as ascorbic acid, vitamin E, the glutathione system (GSH peroxidase, GSH reductase), superoxide dismutase, and catalase work together in the lens to counteract the damaging effects of reactive oxygen species. With age comes a decline in the lens' and aqueous humor's antioxidant system's enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and nonenzymatic (ascorbate, glutathione, cysteine) activity, which may contribute to the formation of senile cataract. Patients with diabetes mellitus have elevated quantities of reactive oxygen species such superoxide anion radicals, whereas enzymatic antioxidant capabilities like superoxide dismutase, catalase, and glutathione peroxidase decrease in these organs. These changes point to a potential involvement for free oxygen radicals and antioxidant systems in diabetic cataract development. Low Cu, Zn-SOD activity were seen in the lenses of diabetes patients with elevated copper ion concentrations. An elevated level of hydrogen peroxide is also associated with diabetes, and the copper ion may cause an exaggerated level of this chemical by inducing a Fenton's reaction with it. The hydroxyl radical (HO) is produced in this process, and further hydroxyl radical (HO) production leads to the formation of the per oxidizing superoxide anion radical.

Diabetes mellitus (DM) is a metabolic illness characterized by impaired or absent insulin sensitivity and production and by abnormalities in glucose metabolism. Microvascular issues such blood vessel sclerosis may lead to myocardial infarction, and diabetes mellitus is closely linked to various disorders, including cardiovascular complications, heart attacks, and obesity. Insulin function may be negatively impacted by an oxidative stress response in the pancreatic cell as a consequence of the metabolic abnormalities seen in diabetes. Insulin resistance in type 2 diabetes patients can be caused by a number of factors, including the activation of NF-kB (nuclear factor-kB) and PKC (protein kinase C), which can disrupt insulin signalling pathways and lead to the production of reactive oxygen species (ROS) like hydrogen peroxide and superoxide anions. It is possible that the deterioration of Islets-cells in the pancreas caused by these organisms contributes to the reduced insulin secretion characteristic of diabetes mellitus.

Chronic hyperglycemia owing to a relative or absolute deficiency in insulin production and/or insulin action, as well as changes in carbohydrate, lipid, and protein metabolism, define the metabolic disorder known as diabetes mellitus. According to the International Diabetes Federation (IFD), 425 million adults throughout the world had

diabetes in 2017. There will be 629 million individuals worldwide with diabetes by 2045, according to estimates. This metabolic disorder is a major public health issue in Morocco. The Moroccan health ministry reports that about 2 million individuals are diabetes. A diabetic's pleading. Nephropathy, neuropathy, and cardiovascular issues are all consequences of uncontrolled diabetes mellitus that may ultimately be fatal. Also, diabetic eye disease is often attributed to the disease. Cataract is the leading cause of preventable blindness worldwide.

Hyperglycemia, caused by a lack of insulin or its ineffective use, is a hallmark of diabetes mellitus (DM), a chronic metabolic disorder. Long-term damage, malfunction, and failure of multiple organ systems, including the eyes, kidneys, nerves, heart, and blood arteries, are related with the chronic hyperglycemia of diabetes. Diabetes Mellitus (DM) is a worldwide pandemic that may be broadly classified into type 1 diabetes (T1D) and type 2 diabetes (T2D). Lack of insulin production due to the destruction of -cells in the pancreas is the hallmark of type 1 diabetes, an autoimmune disease. Multiple factors, including insulin resistance and/or aberrant insulin production, contribute to the development of type 2 diabetes (T2D), a metabolic condition. The prevalence of type 2 diabetes (T2D) is rising rapidly over the world, and this trend is significantly correlated with modifications in dietary and lifestyle habits. From 451 million in 2017, the number of people diagnosed with DM is expected to rise dramatically to 693 million by 2040.

Cataracts are the most common cause of blindness and vision impairment across the globe. Cataracts are more noticeable and develop more quickly in diabetic people. Studies have shown that people with diabetes have a higher-than-normal quantity of thriobarbituric acid-reactive compounds (TBARs), which causes oxidative stress. When there is a discrepancy between ROS production and the antioxidant defense system, oxidative stress may result. In vivo advanced glycation end products (AGEs) development due to hyperglycemia is a result of the Maillard process. Both the oxidative (glycoxidative) and non-oxidative pathways of glycation produce reactive oxygen species (ROS) and ad carbonyl intermediates, making glycation a key source of these compounds. Together, AGEs production events and oxidative processes cause harmful alterations in tissues. Although free radical generation and AGEs creation may be part of the natural ageing process, several researchers have shown a favorable association between AGEs formation, ROS level, cataract development, and diabetes. Reactive oxygen species and glycation may trigger harmful metabolic processes that cause significant damage to the lens. Both free radical generation and the development of AGEs may be slowed by supplementing with antioxidants such a-tocopherol, deferoxamine, or dimethyl sulfoxide.

2. LITERATURE REVIEW

Eva Imelda et.al (2022) Cataracts are the leading cause of visual impairment, accounting for around 47.9% of all cases. Cataracts are associated with both the generation of reactive oxygen species (ROS) and the depletion of endogenous antioxidants. The oxygen in the air creates extremely reactive molecules called ROS. Some types of ROS include peroxides, superoxides, and hydroxyl radicals. Reactive oxygen species (ROS) are generated during oxidative metabolism in the mitochondria, as well as in cellular responses to xenobiotics and bacterial invasion. Lens opacities may advance due to oxidative stress, which can be triggered by high levels of reactive oxygen species (ROS). In their outer orbitals, free radicals like reactive oxygen species (ROS) and other free radicals contain one or more unpaired electrons, making them extremely reactive molecules that may be neutralized by electron-donating chemicals like antioxidants. Vitamin C, vitamin E, and beta-carotene are all examples of antioxidant chemicals found in nature. Multiple studies have shown that antioxidant chemicals found in plants may be utilized to treat or prevent cataracts. These results demonstrate that eating plenty of antioxidant-rich plants is a great way to avoid cataracts, and they raise the possibility of additional research into natural antioxidant extracts for cataract treatment. Numerous additional naturally occurring substances similarly inhibit aldose reductase and stop cataract formation by preventing lens apoptosis.

Alok K. Paul et.al (2022) Novel coronavirus disease 2019 (COVID-19) is caused by the SARS-CoV-2 virus, and is associated with various co-morbidities such as diabetes. Diabetic nephropathy, caused by high blood sugar, is a frequent complication of diabetes. COVID-19 generates serious problems in persons with diabetes mellitus. This article describes how SARS-CoV-2 might exacerbate diabetes-related kidney damage. Diabetes and COVID-19 share important inflammatory mechanisms in their pathogenesis. When SARS-CoV-2 binds to ACE-2, ACE-2 is depleted from blood vessels, which in turn allows angiotensin-II to connect with angiotensin receptor-1 on vascular membranes, leading to the production of NADPH (nicotinamide adenine dinucleotide hydro phosphate), oxidative stress, and vasoconstriction. We anticipate that COVID-19 with co-morbidities like diabetes might synergistically exacerbate oxidative stress, ultimately leading to end-stage renal failure and mortality. Therefore, antioxidants may help protect patients from COVID-19-related comorbidities by lowering the risk of mortality caused by renal impairment caused by oxidative damage. Based on a small number of

patients with COVID-19, a few clinical studies showed how efficient the antioxidant treatment is in improving symptoms. Based on the most up-to-date clinical, preclinical, or in silico investigations, we aimed to evaluate the potential of antioxidants (such as vitamin D and flavonoids) as dietary supplements or therapies against COVID-19 with diabetes as a comorbidity.

Dr. Rahul et.al (2022) Diabetes-induced the risk of blindness due to cataracts is significant in both industrialized and underdeveloped nations. The polyol pathway's significance in the onset of illness has been highlighted in recent fundamental research investigations. Cataracts caused by diabetes are sometimes known simply as "Diabetic cataracts." Cataract risk factors have been characterized thanks in large part to demographic research, which have also improved our understanding of the connection between diabetes and cataract development. Complications during phacoemulsification cataract surgery are more common in diabetic individuals than in the general population. In vitro studies have shown that aldose-reductase inhibitors and antioxidants may help prevent or cure this sight-threatening illness. Important in both the treatment and prevention of diabetic complications, aldose reductase inhibitors are a must-have drug for anybody with diabetes. With more therapeutic potential and fewer adverse effects, natural, herbal, and naturopathic drugs are increasingly being used nowadays. This study delves into the evolution of Eyedrop, a simple, sterile, and safe ocular formulation developed from a standardized extract of an herbal remedy that may be used as an alternative to phacoemulsification. Diabetic cataract may be prevented and treated with the use of a new eyedrop formulation that has undergone standardization and in vitro testing of an effective herbal extract.

Shaidaton Nisha (2022) Cataracts are a leading cause of blindness in people with diabetes in both industrialized and underdeveloped nations. Diabetes-related ocular problems are a leading cause of legal blindness in people with both type 1 and type 2 diabetes. This article seeks to thoroughly appraise data on cataract development in diabetic individuals, cataract types, and the processes underlying cataract pathogenesis. Cataracts cause 33 percent of all cases of vision impairment, according the WHO. Cataract prevalence rose with age in both younger and older diabetics. Rates were greater among women than men. We still don't know what causes cataracts in diabetics, or how to prevent them. Cataract risk factors have been established and our understanding of the connection between diabetes and cataract formation has advanced significantly thanks to population-based research. Obscuration of the lens material, either in the cortex or the posterior subcapsular region, is the defining feature of a simple diabetic cataract. Ocular problems in diabetes are caused by aldose reductase and polyol. In comparison to the research done on other diabetes problems, the mechanisms behind the development of cataracts in diabetics have received less attention. Increased aldose reductase activity has been shown to make a significant impact in both animal and human investigations. After phacoemulsification surgery, many clinical research looked at its effects. There may be a medication for preventing or curing diabetes-related cataracts in the works, as researchers work to produce aldose reductase inhibitors and antioxidants.

Ramith Ramu et.al (2022) The pseudo stem of the banana tree is used in Indian traditional medicine and is also regarded as a healthy food option due to its reputation as an ethnopharmacological agent. This study set out to determine whether banana pseudo stem ethanol extract (EE), Stigmasterol (C1), and -Sitosterol (C2) had any antidiabetic properties. As shown by in vitro tests, both C1 and C2 showed mixed-type inhibition of the -glucosidase enzyme (IC50 values of 7.31 0.12 and 7.80 0.93 g/ml, respectively), with two distinct inhibition constants (K1 and K2). Additional in vitro glycation investigations showed that EE and its constituents blocked intermediate chemical formation and all steps of the protein glycation process. Each of EE, C1, and C2 had an IC50 of 2.67 0.27, 1.80 0.36, and 1.93 0.37 g/ml for aldose reductase inhibition, respectively. Improvements in serum/liver levels and a noteworthy rise in activities of enzymatic and nonenzymatic antioxidants were also seen in in vivo investigations of the diabetes sequelae known as hypercholesterolemia and hypertriacylglycerolemia in diabetic rat models. In conclusion, EE shows promise as a pivotal therapeutic component for addressing diabetes and its consequences.

3. RESEARCH AND METHODOLOGY

Patients above the age of 50 with cataracts who have consented to participate in the study were included. Cataract-free subjects and diabetic cataract subjects were used in this study. Patients at Banjarmasin's Batanghari Hospital and Uline Hospital underwent phacoemulsification-based cataract extraction surgery. Next, a cataract lens was put in 50 mM formalin solution and transferred to the Laboratory of Biochemistry and Biomolecular in the Medical Faculty at Lambing Mangkurat University Banjarbaru, where the SOD and catalase enzyme activities were measured.

The data from the measurements will be tabulated, and the Mann-Whitney test, with a confidence interval of 95%, will be used to conduct statistical analysis.

The fluid was collected at the Ophthalmology Clinic, and it was the result of cleansing the lens capsule's interior after the ejection of the cataract's nucleus during cataract surgery. During the course of three months, 73 cases of senile cataract were gathered. Every specimen obtained was accompanied by detailed patient medical history. The fluid was collected, frozen, and stored at -20°C until the measurements were taken. POCh was where we got our hands on the salts for making the buffer (Gliwice, Poland). Sigma-Aldrich was where we got our adrenaline supply (Milwaukee, USA).

Merck's Folin reagent was used for the protein analysis (Germany). The fluid was thawed, properly mixed, and aliquoted before the protein content and enzyme activity could be measured. The Lowry technique was used to determine protein concentration (Lowry et al., 1951). The adrenaline technique was used to determine the superoxide dismutase activity (Misra, 1985). For this, we used a 1 cm quartz spectrophotometer The data from the measurements will be tabulated, and the Mann-Whitney test, with a confidence interval of 95%, will be used to conduct statistical analysis.

After adding 100 l of a 10 mM adrenalin solution in 10 mM HCl, the reaction began to proceed. The adrenalin underwent spontaneous oxidation at the basic pH, and the kinetics were documented by tracking the rise in absorbance at 480 nm. Adrenaline's oxidation kinetics were measured both with and without the presence of the sample. The rate of adrenalin oxidation inhibition was used to determine Cu,Zn-activity. SOD's This sample's protein concentration was used to determine SOD's relative activity. Student's unpaired t-test was used to determine whether or not there were statistically significant differences between the methods of measuring enzyme activity.

Comparison of superoxide dismutase (SOD) and catalse (catalase) enzyme activity in cataract lenses from August to September 2018 among individuals with and without diabetes mellitus. Table 1 displays the research subject characteristics that emerged from this study.

Homogenat Cataract Lens

To 1 ml of mortar containing the cataract lens and phosphate buffer pH 7.4, the lens was crushed until smooth. After transferring the homogenate to the microtube, centrifuge it at 8000 rpm for 20 minutes. Check the SOD and catalase enzyme activities in the resulting supernatant.

SOD Activity analysis

The supernatant enzyme SOD activity was measured using the Misraand Fridovich technique. 9 SOD is measured in units of the quantity of enzyme required to prevent 50% of the autooxidation of adrenaline. Units per milligram me of protein are used to describe SOD activity.

Catalase Activity analysis

The Aebi technique was used to test the catalase enzyme's activity. 10 The catalase enzyme activity is reported in terms of units per milligram me of protein. The speed is expressed as a constant per second, and one unit is used to do so.

4. DATA ANALYSIS

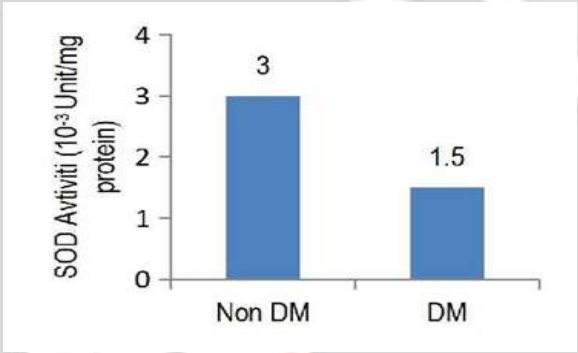
Table 1. Patient and Control Group Characteristics for Studies of Superoxide Dismutase and Catalase Enzyme Activity in Cataract Lenses.

	Characteristic	Non-Diabetes Melitus		Diabetes Melitus	
Age:					
a.	50-59 y.o	4	13,33	8	26,67

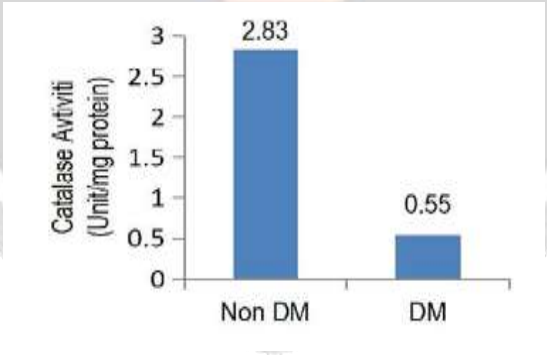
b.	60-69 y.o	8	26,67	7	23,33
c.	≥70 y.o	3	10	-	-
Sex:					
a.	men	9	30	10	33,33
b.	Women	6	20	5	16,67
Blood Glucose Level:					-
a.	< 200	15	50	-	
b.	> 200 (= 316 mg/dL)	-	-	15	50

Table 1 shows that those aged 60–69 make up the largest segment of the population that does not have diabetes mellitus, whereas those aged 50–59 account for the largest segment of the population that does. Those aged 60-69 have the fewest numbers in both the diabetes mellitus and non-diabetes mellitus categories. There seems to be more males than women participating in this study.

Figure 1 displays the results of a statistical analysis of data measuring SOD and catalase enzyme activity (a and b).



(a)



(b)

Figure 1. SOD activity (a) and catalase activity (b) in diabetes mellitus and non-diabetes mellitus groups.

Patients with diabetes mellitus and those without have significantly different levels of SOD and catalase enzyme activity in their cataract lenses, as determined by the Mann-Whitney U test (p=0,000; p0,05). That's because among people with diabetes mellitus, there's an increase in in both diabetic and non-diabetic individuals, reactive oxygen species (ROS) play a role in the pathophysiology of cataract development. Cataracts developed and advanced more rapidly in those with diabetes mellitus than in those without the disease.

The interaction between glucose (an aldehyde) and an amino acid constitutes the non-enzymatic glycation process (protein). 3 Rapid oxidative stress (ROS) and Advanced Glycation End products (AGEs) formation result from this response, which is itself a more rapid type of degeneration. While the glycation process mostly

affects long-lived molecules like proteins, it also has short-lived repercussions, such as the inactivation of active enzymes. That's why, compared to those who don't have diabetes, people with cataracts and diabetes experience more oxidative stress.

This study adds to the growing body of evidence indicating hyperglycemia is a risk factor for cataract formation in the eye lens, since decreased SOD and catalase enzyme activity in cataract lenses is associated with diabetes mellitus. 9 Reduced activities of the antioxidant enzymes SOD and catalase may increase lipid, DNA, and protein modification, which in turn can damage the eye lens and lead to opacity in the lens (cataract).

The samples were assigned to the following independent pairs of categories: primary stages of the cataract (average age of patients 70.5 ± 9.5 years, n = 25) versus mature stages of cataract (average age of patients 69.4 ± 11.3 years, n = 48), and samples from patients without diabetes mellitus versus those obtained from diabetes mellitus patients.

Figure 2 shows the occurrence of measurable Cu,Zn-SOD activity in primary stages of cataract versus frequency of this enzyme activity at mature stages of cataract. It can be seen, that samples with non-detectable activity of Cu,Zn-SOD are substantially more frequent in mature cataract.

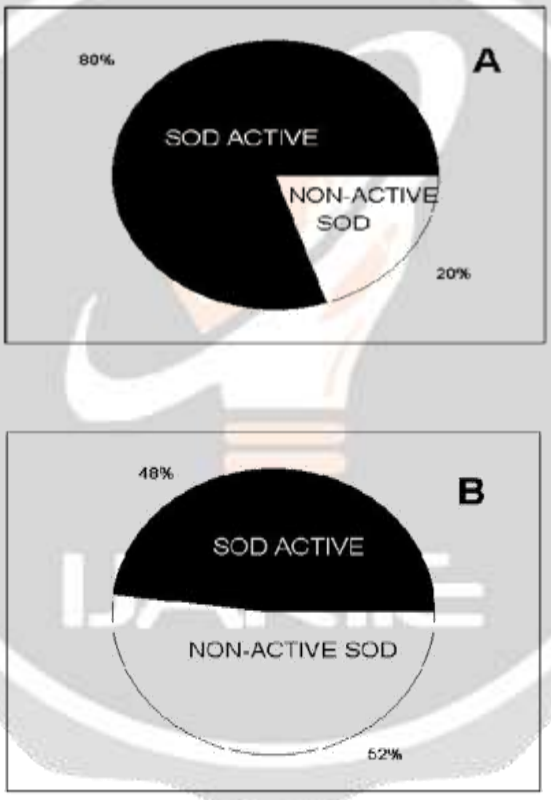


Figure 2. Occurrence of Cu,Zn superoxide dismutase activity in different stages of senile cataract. A. Occurrence of Cu,Zn-SOD activity in primary stages of cataract (n = 19); B. Occurrence of Cu,Zn-SOD activity in ma-ture stages of cataract (n = 54).

As can be shown in Fig. 2A, when cataract and diabetes mellitus occur concurrently, Cu,Zn-SOD tends to be impaired at an earlier stage. Patients with primary stages of cataract but no diabetes mellitus were, on average, 68.4 9.8 years old (n = 12), whereas those with diabetes were, on average, 73.4 8.7 years old (n = 7).

Cataract progression is accompanied by a diminishing difference in Cu,Zn-SOD activity between cataract patients with and without diabetes mellitus (Fig. 2B). Patients with advanced stages of cataract and no diabetes mellitus (n = 36) were younger than those with advanced stages of cataract and diabetic mellitus (n = 12), with a mean age of 69.7 10.6 years. In both Figure 1 and Figure 2, there is a great deal of variety among the groups, which makes it difficult to interpret the data. Table 1 displays information regarding cataract patients' circulatory pathologies, broken down into the two fundamental categories described above.

Table 2. Characteristics of Research Subject of SOD and Catalase Enzymes Activity in Cataract Lenses e with Diabetes Mellitus and Non-Diabetes Mellitus Patients.

Percentage of circulatory system pathologies in cataract patients	(n = 73)
Senile cataract without diabetes mellitus	72.5% (n = 54)
Senile cataract with diabetes mellitus	84.2% (n = 19)

Diabetic mellitus individuals have a much-increased risk of developing circulatory systemdisorders, such as cataracts.

Determining the weak areas of antioxidant defense in cataracts with different etiologies would need more tests of clinical data gathered from individuals with a wide range of medical histories who were treated with a variety of cataract surgical techniques. technical support for gathering research samples.

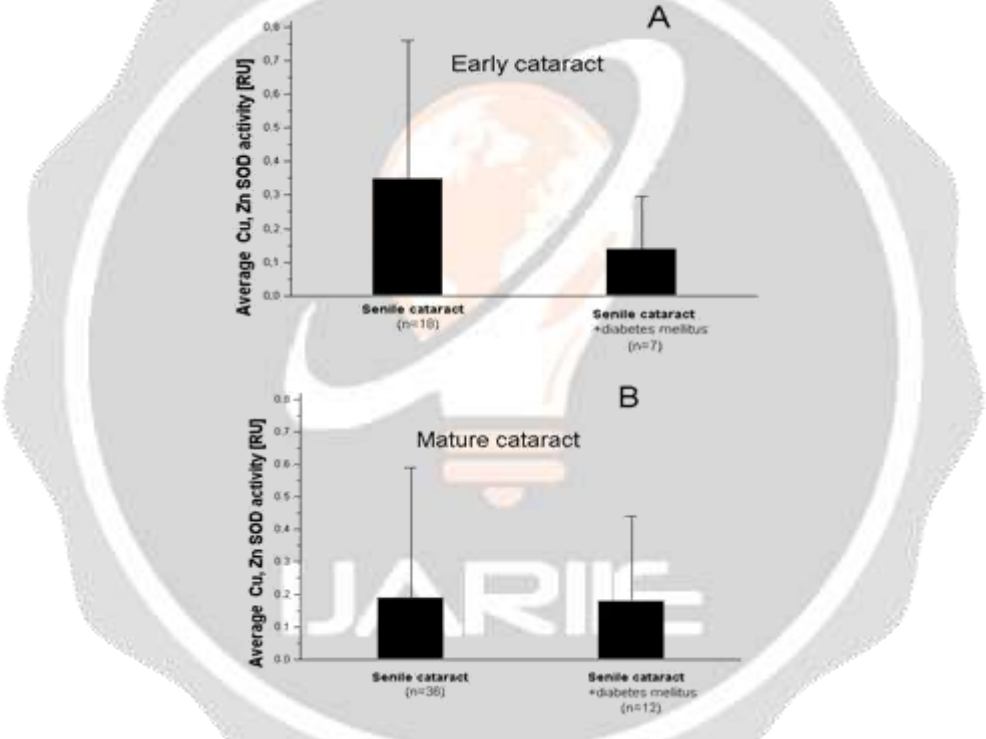


Fig. 3 In individuals with and without diabetes mellitus, average Cu,Zn-SOD activity in senile cataract is shown in Figure 3. Cu,Zn-superoxide dismutase (SOD) activity throughout the early stages of cataract formation (A) and the late stages of cataract development (B).

5. CONCLUSION

Cataract development and diabetes complications are two diseases where oxidative stress is suspected to have a significant impact. The levels of DPP-4 and GLP-1 were shown to be significant in determining the severity of DM in this investigation. In spite of cataract surgeries efficacy, herbal formulation may provide an alternate means of warding off the condition. The retina is a location of significant ROS, LOOH, and lipid aldehyde synthesis because to its high PUFA content, high oxygen consumption, and high degree of metabolic activity. From the above, it may be hypothesized that a diet low in AGE content and high in antioxidant-rich foods may help slow the formation of cataracts, especially in diabetic people. It was determined from this study that the activity of the SOD and catalase enzymes in the cataract lenses of diabetic patients was much lower than that of the non-diabetic individuals studied. The fluid collected from the Ophthalmology Clinic was used to cleanse the lens capsule's interior after the nucleus ejection procedure used to remove a cataract.

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