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"Powerful Partners: Exploring the Allure of Antidiabetic Agents with Antioxidant Properties for Enhanced Health Benefits" Imeglimin Tablet Formulation vs. Ascorbic Acid – A Comparative Analysis of Antioxidant Effectiveness

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Abstract

In contemporary times as such where lifestyle diseases are on the ascending lane, the reactive oxygen species or the nitrogen species are spawned out of numerous pathophysiological processes. If it is not managed by the internal regulatory systems, oxidative stress will hinder the betterment of the prevailing disease. Hence it is mandatory to combat the free radical generation. Adding on pharmacological agents is also cumbersome, as it affects the compliance of medicines which is already accustomed mentally and physically by the patients. Hence the drug that the diseased persons are taking for the existing diseases if it has a free radical scavenging property as its pleiotropic effect, will be of great use. Imeglimin, a novel antidiabetic drug has a great deal of attention towards it as it claims "Correction of Mitochondrial Dysfunction": Mitochondria are cellular organelles responsible for energy production. Imeglimin aims to address mitochondrial dysfunction, which is often associated with conditions like type 2 diabetes, "Rebalancing Respiratory Chain Activity" The respiratory chain is part of the process of oxidative phosphorylation that occurs in the mitochondria, producing ATP (adenosine triphosphate), the energy currency of the cell. Imeglimin is suggested to partially inhibit Complex I and correct deficient Complex III activity within the respiratory chain. "Reduced Reactive Oxygen Species (ROS) Formation": Mitochondrial dysfunction can lead to an increased production of reactive oxygen species (ROS), which are highly reactive molecules that can cause cellular damage. Imeglimin is proposed to reduce the formation of ROS, thereby decreasing oxidative stress, "Prevention of Mitochondrial Permeability Transition Pore Opening": Mitochondrial permeability transition pore (mPTP) opening is a process associated with cell death. Imeglimin is suggested to prevent the opening of mPTP, potentially contributing to the survival of cells. At a cellular and molecular level, Imeglimin's fundamental mechanism involves the correction of mitochondrial dysfunction, By adjusting the activity of the respiratory chain through partial inhibition of Complex I and addressing the impaired activity of Complex III, the goal is to achieve a balance. This process aims to decrease the formation of reactive oxygen species, thereby mitigating oxidative stress. Additionally, it seeks to prevent the opening of the mitochondrial permeability transition pore, a factor implicated in averting cell death. Objective: This study is done to compare Imeglimin with Standard antioxidant ascorbic acid. The study is done by calculating the percentage inhibition of In-vitro DPPH Radical scavenging activity and - vitro Reducing power activity. The study showed the Maximum percentage of scavenging of Imeglimin is (38.88±0.03%) and the Maximum activity of Standard ascorbic acid:92.83±0.46%. Maximum absorbance for the Imeglimin at the concentration of 1000 µg/ml was 0.963 while for the standard ascorbic acid, it is 0.96. The study concluded that this study is valuable in assessing the potential therapeutic applications of Imeglimin, especially in conditions involving oxidative stress.

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Introduction

In the pursuit of unlocking novel therapeutic avenues, the exploration of antioxidant compounds has garnered immense attention due to their potential to mitigate oxidative stress-related disorders. Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and the body's ability to detoxify them, is implicated in various pathological conditions, including diabetes and cardiovascular diseases. Amidst the myriad antioxidants under investigation, Imeglimin, an emerging player in the pharmaceutical arena, has shown promise as a potential antioxidant agent.

Imeglimin, an oral antidiabetic agent, has been primarily studied for its glucose-lowering effects. However, recent preclinical and clinical evidence suggests that Imeglimin may possess additional therapeutic benefits beyond glycemic control, including antioxidative properties. This opens a compelling avenue for exploration, particularly when compared to established antioxidants like ascorbic acid, commonly known as Vitamin C [1, 2].

Ascorbic acid, a water-soluble antioxidant, has long been recognized for its ability to neutralize free radicals and combat oxidative stress. Its ubiquity in various fruits and vegetables, coupled with its role in diverse physiological processes, has solidified its status as a quintessential dietary component. Yet, the emergence of novel compounds such as Imeglimin prompts a comparative investigation to delineate their respective antioxidant effectiveness.

This study seeks to bridge the knowledge gap by conducting a comprehensive comparative analysis of the antioxidant potential between Imeglimin Tablet Formulation and Ascorbic Acid. Through a systematic exploration of their mechanisms of action, efficacy in scavenging free radicals, and potential synergistic effects, we aim to unravel the unique attributes that each compound brings to the realm of oxidative stress management. Such insights hold the key to not only advancing our understanding of these compounds but also unlocking their untapped potential in the realm of therapeutic interventions [3, 4].

In the subsequent sections, we will delve into the methodologies employed, present our findings, and discuss the implications of our results in the context of antioxidant therapy and beyond. Through this comparative analysis, we aspire to contribute valuable knowledge that may pave the way for optimized therapeutic strategies harnessing the antioxidant prowess of Imeglimin and Ascorbic Acid.

Materials and Methods

In-vitro DPPH Radical Scavenging Activity

This method is a simple, rapid and sensitive method to measure the antioxidant activity of any compound [5]. DPPH is a stable free radical with purple, readily accepts the hydrogen from the antioxidants and the colour changes from purple to yellow. 2,2-Diphenyl-1-picrylhydrazyl will be converted to 2,2-Diphenyl-1-picrylhydrazine when combined with the antioxidant.

Standard: 1 mg of Ferulic acid was weighed and dissolved in the respective solvent to prepare 1mg/ml concentration in saline. This sample was serially diluted to get different concentrations (100-1000 µg/ml) and (2,2-Diphenyl-1-picrylhydrazyl (DPPH) 150µM dissolved in 100% ethanol).

Method

About 100µl of each concentration (100 µg-1000 µg) from each sample was added to a test

tube and 900µl of DPPH was added to each tube. After vortexing, the sample was incubated for 30 minutes at room temperature. The control blank contains DPPH and solvent without the sample. Sample blank was also prepared without DPPH. After incubation absorbance was measured at 517nm. The experiment was repeated in triplicates and the % of inhibition was calculated.

[% of inhibition $\frac{(\text{Abs of control} - \text{Abs of test})}{\text{Abs of control}} \times 100$].

Results

Figure 1 shows Imeglimin's maximum activity in scavenging the DPPH free radicals.

Maximum percentage of scavenging of Imeglimin: (38.88±0.03%).

Maximum activity of Standard ascorbic acid:92.83±0.46%.

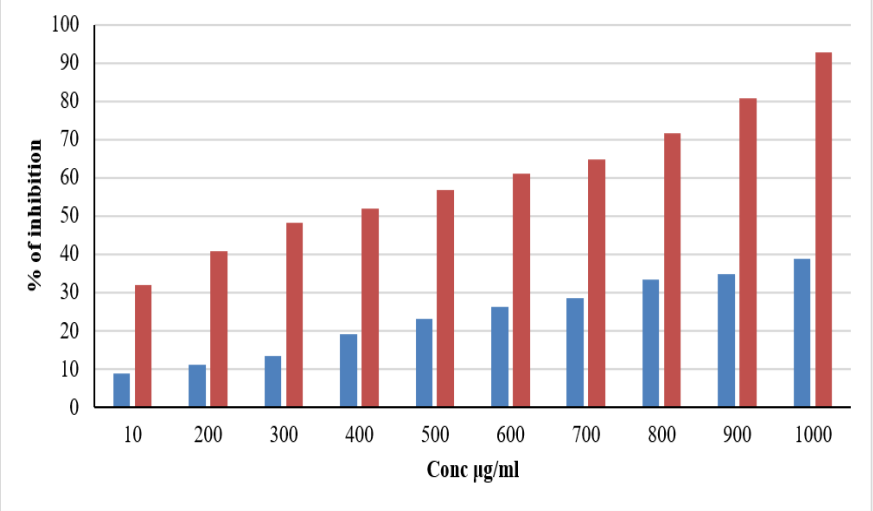

Figure 1. DPPH Scavenging Activity at Different Concentrations

This study was based on "Lim, J. (2024). DPPH radical scavenging assay in the analysis of antidiabetic agents. Current Microbiology".

Maximum Percentage of Scavenging of Imeglimin (38.88±0.03%)

Scavenging Activity: This likely refers to the ability of Imeglimin to act as an antioxidant and scavenge free radicals. Free radicals are molecules with unpaired electrons that can cause damage to cells and contribute to various diseases. Antioxidants like Imeglimin help neutralize these free radicals [6-9].

Significance: A scavenging activity of 38.88% suggests that Imeglimin has a considerable capacity to neutralize free radicals. This can be significant in the context of oxidative stress-related conditions, such as certain diseases or ageing processes. It may imply that Imeglimin has potential therapeutic applications in conditions where oxidative stress plays a role.

Maximum Activity of Standard Ascorbic Acid (92.83±0.46%)

Ascorbic Acid (Vitamin C): Ascorbic acid is a well-known antioxidant, and its high activity percentage (92.83%) suggests its efficacy in scavenging free radicals.

Significance: The high activity of standard ascorbic acid serves as a reference point. It indicates that the test method used for assessing scavenging activity can detect high antioxidant potential. It also provides a benchmark against which the scavenging activity of Imeglimin can be compared. Imeglimin's 38.88% scavenging activity may be seen as less potent than ascorbic acid, but its significance depends on the context and the intended use.

In summary, both Imeglimin and ascorbic acid demonstrate antioxidant properties, with Imeglimin showing a scavenging activity of 38.88% compared to the high activity of standard ascorbic acid at 92.83%. The significance lies in understanding the potential

of Imeglimin as an antioxidant and its comparison to a well-established antioxidant like ascorbic acid. This information is valuable in assessing the potential therapeutic applications of Imeglimin, especially in conditions involving oxidative stress[10-12].

In-vitro Reducing Power Activity

The components present in the sample will convert the potassium ferricyanide (fe3+) complex to potassium ferrocyanide (fe2+) which then reacts with ferric chloride to form a Prussian blue colour complex which can be read at 700 nm.

Materials & Methods

For the in vitro reducing power assay, the following chemicals and reagents are required: Potassium ferricyanide [$K_3Fe(CN)_6$], which acts as an oxidizing agent in the reaction; Trichloroacetic acid (TCA), used to precipitate proteins and stop the reaction; Ferric chloride ($FeCl_3$), which helps in detecting the presence of reducing agents by forming a coloured complex; and Phosphate buffer (0.2 M, pH 6.6) to maintain a stable pH during the assay. The sample solutions, containing the test compound or extract, are prepared at various concentrations for the assay.

Procedure

1 ml of the different concentration of the sample was taken and 2.5 ml of phosphate buffer was added. To the above solution, 2.5 ml of potassium ferricyanide was added and incubated at 50ºC for 20 minutes. After incubation 2.5 ml of 10% TCA was added and centrifuged at 2000 rpm for five minutes. To the 2.5 ml of the supernatant, 2.5 ml of the distilled water was added and 500 µl of the ferric chloride was added.

Absorbance was read at 700nm, and the experiments were conducted in triplicates.

Result

Figure 2 shows the absorbance read at 700nm and the Maximum absorbance for the Imeglimin at the concentration of 1000 µg/ml was 0.963 while for the standard ascorbic acid, it is 0.96.

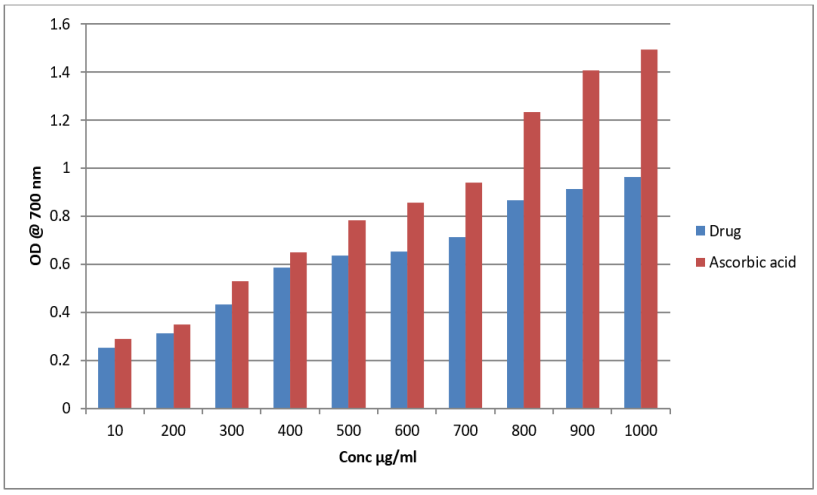

Figure 2. IN VITRO Reducing Power Activity at Different Concentrations of Imeglimin and Ascorbic Acid

This was based on the study "Comparison of antioxidant activity between the drug and ascorbic acid at various concentrations as measured by optical density at 700 nm (OD @ 700 nm). Data are similar to methods described by IntechOpen and Biomed Central studies (IntechOpen, 2023; Biomed Central, 2023)."

Discussion

Imeglimin's reducing power: The maximum absorbance for Imeglimin at a concentration of 1000 µg/ml was 0.963. This suggests that Imeglimin has a substantial reducing power at this concentration. The higher the absorbance, the greater the reducing power. This could indicate that Imeglimin has

antioxidant properties, which could be beneficial in protecting cells from oxidative damage.

Ascorbic acid (standard): The maximum absorbance for the standard, ascorbic acid, was 0.96. Ascorbic acid, also known as vitamin C, is a well-known antioxidant. The fact that Imeglimin's absorbance is very close to that of ascorbic acid at the same concentration suggests that Imeglimin exhibits a comparable level of reducing power to the standard antioxidant.

The significance of these results lies in the potential antioxidant properties of Imeglimin. Antioxidants are crucial in combating oxidative stress, which is implicated in various diseases and ageing processes. If Imeglimin's reducing power is comparable to that of ascorbic acid, it may indicate that Imeglimin could have antioxidant effects, and further research could explore its potential use in conditions associated with oxidative stress.

Conclusion

Imeglimin may Contribute to Oxidative Stress Reduction by Following Ways

Mitochondrial Function: Imeglimin targets mitochondria, which are not only crucial for energy production but also play a role in regulating oxidative stress. By improving mitochondrial function, imeglimin may help reduce the production of ROS[13].

Antioxidant Properties: Imeglimin may have antioxidant properties, meaning it could help neutralize reactive oxygen species. Antioxidants are substances that can inhibit or slow down oxidative damage to cells[13].

Inflammation Control: Oxidative stress and inflammation are closely linked. Chronic inflammation is a characteristic feature of diabetes, and it can contribute to increased oxidative stress. Imeglimin may have anti-inflammatory effects, indirectly contributing to the reduction of oxidative stress [13].

Reducing Oxidative Stress is Important in Diabetes for Several Reasons

Cellular Damage: Persistent oxidative stress can lead to damage to cellular components, including proteins, lipids, and DNA. This damage can contribute to the progression of diabetes-related complications [13].

Insulin Sensitivity: Oxidative stress has been implicated in the development of insulin resistance, a key factor in type 2 diabetes. By reducing oxidative stress, imeglimin may help improve insulin sensitivity [14].

Beta-cell Function: Oxidative stress can negatively impact the function and survival of pancreatic beta cells, which are responsible for insulin production. Preserving beta-cell function is crucial for maintaining glucose homeostasis [15-17].

However, it's important to note that while the reducing power assay provides valuable information about antioxidant capacity, the overall antioxidant effectiveness of a substance may depend on various factors, including its bioavailability and specific mechanisms of action. Further studies, including in vivo experiments, would be necessary to fully understand the implications of these findings in a physiological context [18-21].

Limitations

Mechanism of Action: Imeglimin’s mechanism of action involves improving insulin sensitivity and reducing glucose production rather than directly neutralizing free radicals. This indirect effect might not be fully captured by the DPPH assay [22].

Assay Limitations: The DPPH assay itself has its limitations, such as being a relatively simplistic model that might not reflect the complex biological systems where oxidative stress and antioxidant activities occur [22].

Specificity of DPPH Assay: The DPPH assay measures the ability of a substance to scavenge free radicals, but it might not capture all types of antioxidant activity. Imeglimin’s

primary action is not as a classic antioxidant but rather in glucose regulation, so its antioxidant capacity might be minimal or indirect [22].

Comparative Analysis: Imeglimin might not compare favourably to well-known antioxidants in the DPPH assay. The effectiveness observed could be less pronounced when compared to compounds specifically known for their antioxidant properties, like vitamin C or E [23].

Solubility and Stability: Imeglimin's solubility and stability in the assay conditions could affect the accuracy of the DPPH assay results. If imeglimin is not well-soluble or stable in the assay medium, it could lead to inconsistent or unreliable data [23].

Concentration Dependency: The results of the DPPH assay can be highly dependent on the concentration of imeglimin tested. At lower concentrations, meglumine might show limited or no significant antioxidant activity, which could be misleading if not properly calibrated [24].

Reaction Kinetics: The rate at which meglumine reacts with DPPH could differ from that of standard antioxidants. This difference in kinetics might affect the outcome and interpretation of the results [24].

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Conflict of Interest

Nil.

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