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(54) **Title:** THERAPEUTIC FORMULATION COMPRISING OF AN ALPHAGLUCOSIDASE INHIBITOR AND NITRIC OXIDE RELEASING MOIETY COMPLEXATED WITH A NITRIC OXIDE SCAVENGER

(57) **Abstract:** A therapeutic formulation comprising of Alphaglucosidase Inhibitors as active agents namely, Acarbose and Miglitol, characterized in that said Alphaglucosidase Inhibitors are combined or complexated with NO Scavenger to prevent Diabetic Complications arising out of Nitrosative stress.

TITLE: THERAPEUTIC FORMULATION COMPRISING OF AN ALPHAGLUCOSIDASE INHIBITOR AND NITRIC OXIDE RELEASING MOIETY COMPLEXATED WITH A NITRIC OXIDE SCAVENGER

Field of Invention

The present invention belongs to the field of pharmaceutical technology and relates to a pharmaceutical composition that aims at combating secondary complications in diabetes drug intake and optimizes the therapeutic usefulness of Oral Hypoglycemic Agents called Alphaglucosidase Inhibitors, which would include Acarbose and Miglitol and comprises of the unique advantage of Nitric Oxide Releasing moiety, which is further complexated with a Nitric Oxide Scavenger. The therapeutic edge of this invention would be minimizing the risk of Diabetic Complications due to the Oxidative Cellular Damage produced by Nitric Oxide. Besides, Nitric Oxide Scavenger has been complexated to reduce the NO levels and hence enhance the safety profile of the products on a long-term use.

Background of the Invention

The most important causes of Diabetic Complications appear to be: a) Auto-oxidation of Glucose; b) Induction and activation of various Lipoxygenase enzymes; c) Activation of Glycation pathways; d) Promotion of the interaction of the Nitric Oxide with superoxide anions to produce peroxynitrite and hydroxyl radicals and e) Reduction of the activity of the Antioxidant defense mechanisms. The major attributing factor for this large scale damage has been identified as Nitric Oxide,

one of the most important biological molecules, which is capable of producing several cellular responses, both beneficial as well as detrimental.

Metabolic Disorders with an inevitable nexus with Cardiovascular Diseases, pose one of the biggest threat to the Scientific Community, contributing to one of the highest number of deaths in populations across the globe. The most important triggering factor appears to be the changes in the lifestyle and dietary pattern, for the onset of the disease in the individuals. However, the secondary complications are primarily due to factors that are quite unlikely to be noticed. It's precisely because of this reason that Diabetes is often referred to as a "Silent Killer", with a potential damage to the vital organs.

Macro- and microvascular diseases are the most common causes of morbidity and mortality in patients with diabetes mellitus. Diabetic cardiovascular dysfunction represents a problem of great clinical importance underlying the development of various severe complications including retinopathy, nephropathy, neuropathy and increase the risk of stroke, hypertension and myocardial infarction. Hyperglycemic episodes, which complicate even well controlled cases of diabetes, are closely associated with increased oxidative and nitrosative stress, which can trigger the development of diabetic complications. Hyperglycemia stimulates the production of advanced glycosylated end products, activates protein kinase C, and enhances the polyol pathway leading to increased Superoxide anion formation. Superoxide anion interacts with nitric oxide, forming the potent cytotoxin peroxynitrite, which attacks various biomolecules in the vascular endothelium, vascular smooth

muscle and myocardium, leading to cardiovascular dysfunction. The pathogenetic role of nitrosative stress and peroxynitrite, and downstream mechanisms including poly(ADP-ribose) polymerase (PARP) activation, is not limited to the diabetes-induced cardiovascular dysfunction, but also contributes to the development and progression of diabetic nephropathy, retinopathy and neuropathy. Accordingly, neutralization of peroxynitrite or pharmacological inhibition of PARP is a promising new approach in the therapy and prevention of diabetic complications. Thus it would be important to determine the role of nitrosative stress and downstream mechanisms including activation of PARP in diabetic complications and evolve novel therapeutic strategies such as neutralization of peroxynitrite and inhibition of PARP

Evidence implicates hyperglycemia-derived oxygen free radicals as mediators of diabetic complications. However, intervention studies with classic antioxidants, such as vitamin E, failed to demonstrate any beneficial effect. Recent studies demonstrate that a single hyperglycemia-induced process of overproduction of Superoxide by the mitochondrial electron-transport chain seems to be the first and key event in the activation of all other pathways involved in the pathogenesis of diabetic complications. These include increased polyol pathway flux, increased advanced glycosylation end product formation, activation of protein kinase C, and increased hexosamine pathway flux. Superoxide overproduction is accompanied by increased nitric oxide generation; due to an endothelial NOS and inducible NOS uncoupled state, a phenomenon favoring the formation of the strong oxidant peroxynitrite, which in turn damages DNA. DNA damage is an obligatory stimulus for the activation of the nuclear enzyme poly(ADP-ribose) polymerase.

Poly(ADP-ribose) polymerase activation in turn depletes the intracellular concentration of its substrate NAD+, slowing the rate of glycolysis, electron transport, and ATP formation, and produces an ADP-ribosylation of the GAPDH. These processes result in acute endothelial dysfunction in diabetic blood vessels that, convincingly, also contributes to the development of diabetic complications. These new findings may explain why classic antioxidants, such as vitamin E, which work by scavenging already-formed toxic oxidation products, have failed to show beneficial effects on diabetic complications and may suggest new and attractive "causal" antioxidant therapy. New low–molecular mass compounds that act as SOD or catalase mimetics or L-propionyl-carnitine and lipoic acid, which work as intracellular Superoxide scavengers, improving mitochondrial function and reducing DNA damage, may be good candidates for such a strategy, and preliminary studies support this hypothesis. This "causal" therapy would also be associated with other promising tools such as LY 333531, PJ34, and FP15, which block the protein kinase ß isoform, poly(ADP-ribose) polymerase, and peroxynitrite, respectively.

Insulin action is initiated through its binding to the cell surface receptor, initiating a series of signal transduction reactions, which stimulate various effectors to produce its physiological effects. Therefore, impairment of insulin signal transduction results in attenuation of insulin action and leads to insulin resistance resulting in type 2 diabetes mellitus. Because the molecular mechanisms of insulin resistance are still being elucidated, it is indispensable to establish in vitro models of basal and insulin-mediated signal transduction to clarify these mechanisms and suggest treatments where appropriate.

Evidence demonstrates that exogenously administered nitric oxide (NO) can induce insulin resistance in skeletal muscle. The modulatory effects of two NO donors, S-nitroso-N-acetyl D, L-penicillamine (SNAP) and S-nitrosoglutathione (GSNO) were investigated on the early events in insulin signaling in rat skeletal myocytes. Skeletal muscle is responsible for about 75% of whole body glucose metabolism, and insulin resistance is a characteristic feature of individuals with type II diabetes mellitus. A number of intracellular defects in insulin action in muscle have been described, including decreased glucose transport and glucose phosphorylation and diminished glycogen synthase activity. A similar effect is observed in rodent model systems. It was also noted that acute treatment of skeletal myocytes by either GSNO or SNAP resulted in significantly reduced content of available IR-β for participating in insulin-mediate signal transduction. This could be a possible explanation for the decrease in insulin binding and insulin receptor sites observed in mononuclear leukocytes and erythocytes treated with these NO donors

There is increasing evidence that endogenous nitric oxide (NO) influences adipogenesis, lipolysis and insulin stimulated glucose uptake.

Endothelium derived nitric oxide is synthesized from the amino acid L-arginine by the endothelial isoform of nitric oxide synthase (NOS), yielding L-citrulline as a byproduct. Nitric oxide is labile with a short half-life (< 4 seconds in biological solutions). It is rapidly oxidised to nitrite and then nitrate by oxygenated haemoglobin before being excreted into the urine. Several co-factors are required for nitric oxide biosynthesis. These include nicotinamide adenine dinucleotide phosphate (NADPH),

flavin mononucleotide, flavin adenine dinucleotide, tetrahydrobiopterin (BH4), and calmodulin. Once synthesised, the nitric oxide diffuses across the endothelial cell membrane and enters the vascular smooth muscle cells where it activates guanylate cyclase, leading to an increase in intracellular cyclic Guanosine-3',5-Monophosphate (cGMP) concentrations4. As a second messenger, cGMP mediates many of the biological effects of nitric oxide including the control of vascular tone and platelet function. In addition, nitric oxide has other molecular targets, which include haem, or other iron centred proteins, DNA, and thiols. These additional reactions may mediate changes in functions of certain key enzymes or ion channels. Nitric oxide also interacts with enzymes of the respiratory chain including complex I and II, and aconitase, and through these effects alters tissue mitochondrial respiration. Interaction of nitric oxide with superoxide anion can attenuate physiological responses mediated by nitric oxide and produce irreversible inhibitory effects on mitochondrial function as a result of the formation of peroxynitrite (ONOO−), a powerful oxidant species.

NOSs are the only enzymes known to simultaneously require five bound cofactors/prosthetic groups: FAD, FMN, haem, tetrahydrobiopterin (BH4) and Ca2+-calmodulin (CaM). In mammals, three distinct genes encode NOS isozymes: neuronal (nNOS or NOS1), cytokine inducible (iNOS or NOS2) and endothelial (eNOS or NOS3).

The essential role of nitric oxide (NO) in normal physiology and its involvement in the pathophysiology of a variety of diseases render the compound an attractive therapeutic target. NO donor drugs are used in the treatment of hypotension and angina where abnormalities in the L-

arginine-nitric oxide pathway have been implicated. Overproduction of NO has been associated with a number of disease states including septic shock, inflammatory diseases, diabetes and its complications, ischaemia-reperfusion injury, adult respiratory distress syndrome, neurodegenerative diseases and allograft rejection. NO is produced by a group of enzymes, the nitric oxide synthases. Selective inhibition of the inducible isoform is one approach to the treatment of diseases where there is an overproduction of NO; an alternative approach is to scavenge or remove excess NO. A number of NO scavenger molecules have demonstrated pharmacological activity in disease models, particularly models of septic shock. These include organic molecules such as PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), haemoglobin derivatives such as the pyridoxalated haemoglobin polyoxyethylene conjugate (PHP), low molecular weight iron compounds of diethylenetriaminepentaacetic acid and diethyldithiocarbamate and ruthenium polyaminocarboxylate complexes. The data suggest a potential role for NO scavengers in the treatment of NO mediated disease

Thus, while treating Diabetes, prevention of macro and microvascular complications is of paramount importance and therefore, Oral Hypoglycemic Agents such as Sulfonylureas and Biguanides alone may not suffice. The augmentation with Nitric Oxide Scavengers would ensure greater protection to the vasculature from the potential damage caused by a cascade of events following pronounced nitrosative stress. It's well known that conventionally used antioxidants do not have any perceptible role in neutralising the nitrosative stress and the Superoxide Anion.

However, since NO is a double edged weapon, it's important to guard against the deleterious effects. Hence it would be prudent to combine a NO-scaveger along with the above anti diabetic drug to ensure long-term safety of the products.

Objectives

An Objective of this invention is to treat Diabetes and prevent its complications by providing the Oral Hypoglycemic Agents, commonly known as, "Alphaglucosidase Inhibitors". The Invention embodies in itself the Claim for separate and individual drugs, that would include Acarbose and Miglitol as separate entities and separate set of inventions

Another Objective of this invention is to prevent the Diabetic complications produced due to excessive nitrosative stress by mitigating the deleterious effects of NO, with the combination/complexation of the Antidiabeti drugs with the NO scavengers, which would include PTIO, PHP, low molecular weight iron compounds of diethylenetriaminepentaacetic acid, diethyldithiocarbamate or ruthenium polyaminocarboxylate complexes or any other suitable NO scavenger

Another objective of this invention is to develop useful and convenient dosage levels and forms of such a combination therapeutic.

Yet another objective of this invention is to enhance individual effects of the components of this composition by their combination.

Summary

The invention embodies in itself 2 separate entities, each belonging to the class of drugs known as "Alphaglucosidase Inhibitors". Further, each active ingredient from the said class has been combined or complexated with NO Scavenger to prevent Diabetic Complications arising out of Nitrosative stress

The active ingredients are Alphaglucosidase Inhibitors, which block the action of alpha-glucosidase enzymes at the brush border of the intestine.

The inactive ingredients are more or less the same for each of the compound: microcrystalline cellulose or hydroxypropyl methylcellulose, colloidal silicon dioxide, Magnesium stearate, Sodium, Starch, PEG and other appropriate agents

Detailed Description of the invention

The inhibition by the Alphaglucosidase Inhibitors slows the breakdown of dietary oligosaccharides and disaccharides. The delayed digestion of carbohydrates decreases post-prandial glucose concentrations. As monotherapy, alpha-glucosidase inhibitors lower FPG by 20 to 30 mg/dl and hemoglobin A_{1c} by 0.7 to 1%. Additionally, acarbose and miglitol decrease post-prandial glucose by 30 to 70 mg/dl. Acarbose and Miglitol have minimal effect on cholesterol and body weight.

The compositions are as follows:

1. Acarbose 25 to 100 mg per day in combination or complexated with NO-Scavenger
2. Miglitol 25 to 100 mg per day in combination or complexated with NO-Scavenger

Disclosure of Invention

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

I claim:

1. A therapeutic formulation comprising of Alphaglucosidase Inhibitors as active agents namely, Acarbose and Miglitol, characterized in that said Alphaglucosidase Inhibitors are combined or complexated with NO Scavenger to prevent Diabetic Complications arising out of Nitrosative stress.
2. A therapeutic formulation as claimed in Claim 1 wherein NO scavenger would include PTIO, PHP, low molecular weight iron compounds of diethylenetriaminepentaacetic acid, diethyldithiocarbamate or ruthenium polyaminocarboxylate complexes.
3. A therapeutic formulation as claimed in Claim 1 wherein microcrystalline cellulose or hydroxypropyl methylcellulose, colloidal silicon dioxide, Magnesium stearate, Sodium, Starch, PEG and other appropriate agents are added to the said Alphaglucosidase Inhibitors as inactive agent.
4. A therapeutic formulation as claimed in Claim 1 wherein Acarbose is combined or complexated with NO-Scavenger in a dosage range of 25 to 100 mg per day.
5. A therapeutic formulation as claimed in Claim 1 wherein Miglitol is combined or complexated with NO-Scavenger in a dosage range of 25 to 100 mg per day.