Effect of Vitamin D With and Without Acipimox on Oxidative stress in Diabetes

Ayasha Saiffi[1*], Sokindra kumar[1], Lubhan Singh[1]

[1]*Faculty of* Pharmacy, Swami Vivekanand Subharti University, Meerut-250005
*Corresponding author:Ayasha Saiffi
Department of Pharmacology,Kharvel Shubharti College of Pharmacy,
Swami Vivekanand Subharti University,
Meerut, 250005, India.
ayashasaiffi@gmail.com

ABSTRACT:

Diabetes mellitus (DM) is a disease associated with metabolism which is characterized through hyperglycemia, ensuing in impaired insulin secretion and/or action. DM can be classified into two main classes: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), with T2DM affecting about 5% of the population. T2DM is associated with long-term organ damage, dysfunction and failure, and is currently one of the costliest chronic diseases in the world (ADA - American Diabetes Association (ADA), 2011. This disease is also linked to obesity, insulin resistance, and defects in pancreatic β-cell function and mass. The diabetic patients has been partly linked with the consumption of a high-calorie diet and sedentary lifestyle which results diabetes due to obesity. T1D is an autoimmune multifactorial disease in which genetic predispositions are associated with destruction of the β cells of the pancreas which results in the lack of insulin secretion. The Disease can occur at any age childhood and adolescence. An unknown environmental factor triggers β-cell-specific autoimmunity event where viruses, bad life style, bacteria and diet have all been implicated. T2DM is the most popular type of the diabetes and is characterized by diminished peripheral insulin sensitivity, lack of efficient glucose uptake in targeted tissues such as adipose tissue, skeletal muscle and adipose tissue, impaired regulation of hepatic glucose production, and declining β-cell function. These metabolic defects lead to β cell failure. Hyperglycemic state occurs when insulin secretion is unable to recompense for insulin resistance. T2DM is amplified by several genetic component and environmental factors such as age, obesity, diet, and lack of physical activity. Both type of diabetes i.e., T1D and T2D are associated with an increased risk of vascular and metabolic disorders. The present study investigated the effects of Acipimox and Vitamin D as on High fat diet –induced diabetes in rats. Various biochemical parameters such as SOD, GSH and catalase activity were also assessed. The present study demonstrates that Acipimox had potential therapeutic effects on diabetes in rats via inhibiting oxidative stress.

KEYWORDS: Vitamin D, Acipimox, antioxidant, Diabetes, oxidative stress

INTRODUCTION:

Obesity and insulin resistance are usually as a result of an immoderate dietary circumstance related to an unbalanced power intake, expenditure, and storage[1]. Importantly, through secreting numerous humoral substances and/or neuronal networks, the liver and adipose tissue work together to maintain the balance of glucose and lipids[2]. To find out which initial events trigger the development of High Fat Diet-induced insulin resistance and obesity, we globally determine the biological pathways that are coordinately altered in both the liver and adipose tissue of rat feed With high fat diet[3]. We found that oxidative stress pathways, which are regulated through the balance of reactive oxygen species (ROS) production and antioxidant enzyme activity , are up-regulated in both tissues before the onset of insulin resistance and obesity induced by an HFD[4]. Diabetes is a chronic health condition characterized by high levels of sugar (glucose) in the blood[5]. It occurs when the body either does not produce enough insulin or cannot effectively use the insulin it produces[6]. Insulin is a hormone that regulates the metabolism of carbohydrates and helps in the uptake of glucose by cells to use as energy[7].

There are several types of diabetes, including:

1. Type 1 Diabetes: This type of diabetes is an autoimmune disease in which the immune system mistakenly attacks and destroys the insulin-producing cells in the pancreas[8]. People with type 1 diabetes require daily insulin injections or the use of an insulin pump to survive[9]. It usually develops in childhood or adolescence, but it can occur at any age[10].

2. Type 2 Diabetes: Type 2 diabetes is the most common form of diabetes, accounting for the majority of cases[11]. It occurs when the body becomes resistant to the effects of insulin or does not produce enough insulin to maintain normal blood sugar levels[12]. It is often associated with lifestyle factors such as obesity, sedentary lifestyle, and poor diet. Type 2 diabetes can often be managed through lifestyle changes, such as adopting a healthy diet, regular physical activity, and sometimes medication or insulin therapy[13].

3. Gestational Diabetes: Gestational diabetes develops during pregnancy and affects some women who have never had diabetes before[14]. It usually disappears after childbirth, but women who have had gestational diabetes are at an increased risk of developing type 2 diabetes later in life[15].

The common symptoms of diabetes include excessive thirst, frequent urination, unexplained weight loss, increased hunger, fatigue, blurred vision, slow wound healing, and frequent infections[9]. However, some people with type 2 diabetes may not experience noticeable symptoms in the early stages.

If you suspect you may have diabetes or are experiencing any symptoms, it's important to consult a healthcare professional for proper diagnosis and management[16]. Diabetes requires ongoing monitoring and treatment to prevent complications that can

affect various organs and systems in the body, including the heart, blood vessels, kidneys, eyes, and nerves[17].

Managing diabetes typically involves maintaining healthy blood sugar levels through a combination of medication (such as insulin or oral medications), lifestyle changes (such as a balanced diet, regular exercise, and weight management), monitoring blood sugar levels, and regular check-ups with healthcare providers. It's essential to work closely with a healthcare team to develop an individualized diabetes management plan[5].

MATERIALS AND METHODS:

Animals: Animals were obtained from animal house facility of AIIMS, New Delhi. The animal were acclimatized for one week then divided into the six groups (n=6), and maintained on rats fed with normal pellet diet and water *ad libitum.*All the animals had kept with good and standard laboratory condition like light (12 h light ;12 h dark) at controlled room temperature $25\pm2\ ^0$ C and at maintained relative humidity (50±15).

Drugs: Acipimox (Brand Name: Acipicap) Vitamin D (Brand Name: Uprise d3) Metformin (Brand Name: Glyciphage)

Study design: Institutional Animal Ethics Committee permission, the study was conducted in Wistar rats (200-250 g). The animal model was developed using a high fat oral diet (vanaspati ghee: coconut oil,3:1) along side 25% fructose (excessive sugar) delivered in consuming water over a length of 6 weeks. Rats are divided into 6 groups as follows:

Group 1: Normal control, Animals received standard pellet diet and purified water for 24 h over a period of 6weeks and was considered as normal control (NC) group.

Group 2: HFD Group, Animals received standard pellet diet, 25% fructose water in bottles for 24 h and 3 ml excessive fat feed every day over a length of 6 weeks.

Group 3: HFD+Vit D, Animals received standard pellet diet, 25% fructose in bottles for 24 h and 3 ml high fat feed every day over a length of 6 weeks. After 3 weeks of study, Vitamin D at a dose of 500 IU/kg/day was administered after 3 weeks of feeding fat diet, and was continued until remain-ing 3 weeks.

Group 4: HFD+Acipimox, Animals received standard pellet diet, 25% fructose in bottles for 24 h and 3 ml high fatdiet each day over a period of 6 weeks. After 3 weeks of study, Acipimox at a dose of 50 mg/kg/day was administered after 3 weeks of feeding fat diet, and was continued until remain-ing 3 weeks.

Group 5: HFD+Vit D+Acipimox, Animals received standard pellet diet, 25% fructose in bottles for 24 h and 3 ml high fatdiet each day over a period of 6 weeks. After 3 weeks of study, Acipimox and Vitamin D was administered after 3 weeks of feeding fat diet, and was continued until remain-ing 3 weeks

Group 6: STANDARD, Animals received standard pellet diet, 25% fructose in bottles for 24 h and 3 ml high fat diet each day over a period of 6 weeks. After 3 weeks of study Metformin (100mg/kg/day) was administered after 3 weeks of feeding HFHS diet, and was continued until 3weeks .

Measurement of Oxidative Stress

Serum MDA, SOD levels and catalase activity from serum were evaluated to assess the oxidative stressdeveloped in the model.

Superoxide dismutase (SOD) measurement: SOD activity was measured based on the ability of the enzyme to inhibit the autoxidation process of pyrogallol. A modification of the procedure described by Marklund and Marklund was adopted for assay of SOD activity(9). Briefly, the tissues were homogenised in 50 mmol/L phosphate buffer (pH 7.8) using a Polytron homogeniser. The homogenate was centrifuged at 1,600 g for 15 minutes. 20 µl of 10 mmol/L of pyrogallol solution was added to various concentrations of the tissue supernatants and the rate of autoxidation was measured spectrophotometrically at 420 nm. SOD activity is expressed as units of SOD/mg protein (1.0 U is defined as the amount of the enzyme, which causes 50% inhibition of pyrogallol autoxidation).
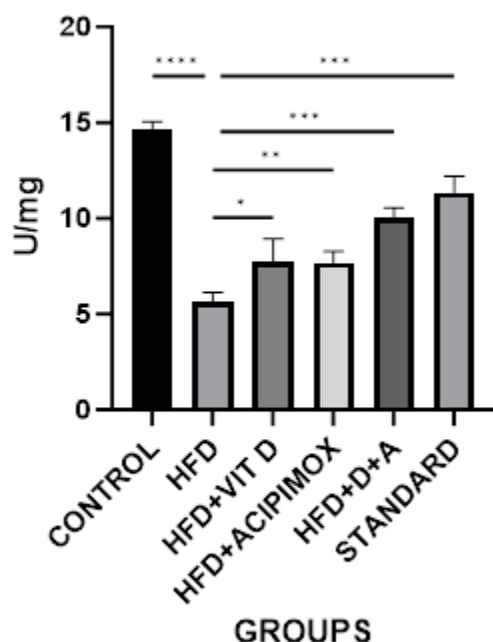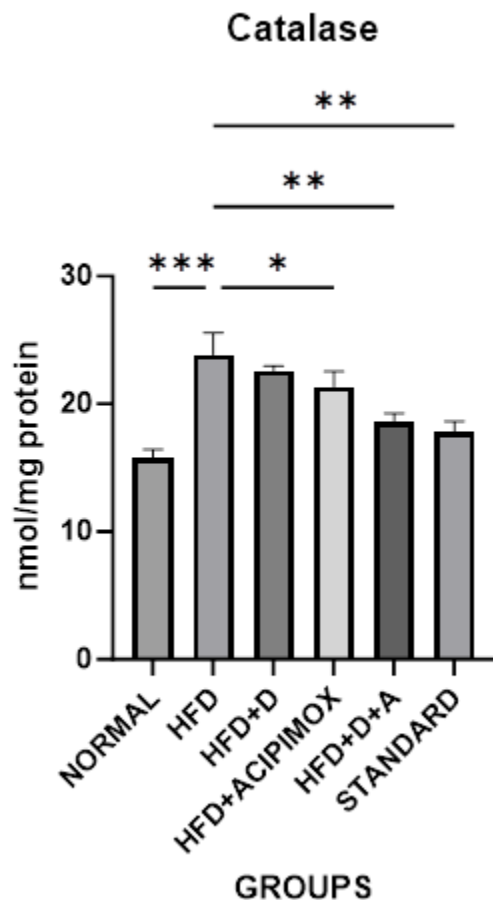

Figure 1.Data represent as mean ± SD; Effect of vitamin D and Acipimox on oxidative parameters (N=6 animals/group) against high fat diet induce diabetes on wistar rats. Data analyses was performed using one way ANOVA. *P<0.05 .,P<0.001,. control vs induced and induced vs treatment group.**

Catalase (CAT) measurement: CAT activity primarily based totally at the capacity of the enzyme to interrupt down H2O2. The technique of Aebi became hired withinside the assay of CAT activity(10).

Briefly, the tissues have been homogenised in isotonic buffer (pH 7.4). The homogenate become centrifuged at 1,000 g for 10 minutes. 20 µl of 100-fold diluted tissue supernatant was added to 980 µl of the assay

mixture containing 900 µl of 10 mmol/L of H_2O_2, 50 µl of Tris HCI buffer (pH 8.0) and 30 µl of distilled water. The rate at which decomposition of Hydrogen Peroxide occur is was measured spectrophotometrically at absorbance 240 nm. CAT activity is expressed as k/mg protein, where k is the first order kinetic rate constant.
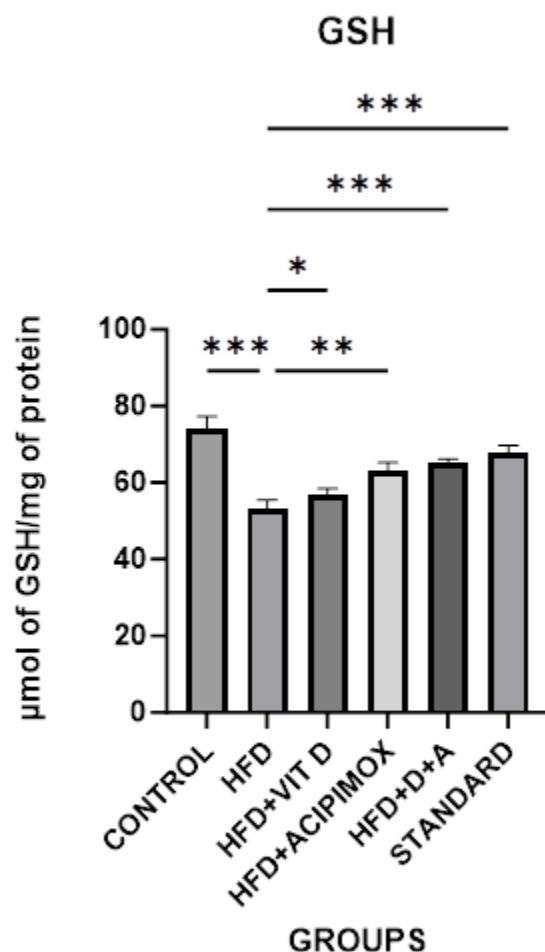

Figure 2.Data represent as mean ± SD; Effect of vitamin D and Acipimox on oxidative parameters (N=6 animals/group) against high fat diet induce diabetes on wistar rats. Data analyses was performed using one way ANOVA. *P<0.05 .,P<0.001,. control vs induced and induced vs treatment group.**

Reduced glutathione (GSH) measurement: Reduced glutathione levels were estimated based on the ability of the SH group to reduce 5,5'-dithiobis- (2-nitrobenzoic acid) to form 1 mole of 2-nitro-5- mercaptobenzoic acid per mole of SH. The method of Sedlak and Lindsay was employed in the determination of GSH levels(11). Briefly, the tissues were homogenised in 50 mmol/L Tris HCI buffer (pH 7.4). The homogenate has been centrifuged sequentially at 10,000 g for 20 minutes, then at 100,000 g for 60 minutes. To 0.5 ml of tissue supernatant, 1.5 ml of 0.2 mol/L Tris HCI buffer (20 mmol/L EDTA, pH 8.2), 0.1 ml of 0.01 mol/L of

5,5'-dithiobis-(2-nitrobenzoic acid) and 7.9 ml of methanol were added. The mixture was incubated at 37ºC with occasional shaking for 30 minutes. The mixture was then centrifuged at 3,000 g 15 minutes and the absorbance of the supernatant was determined at 412 nm. The GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH. GSH levels are expressed as µmol/mg protein.
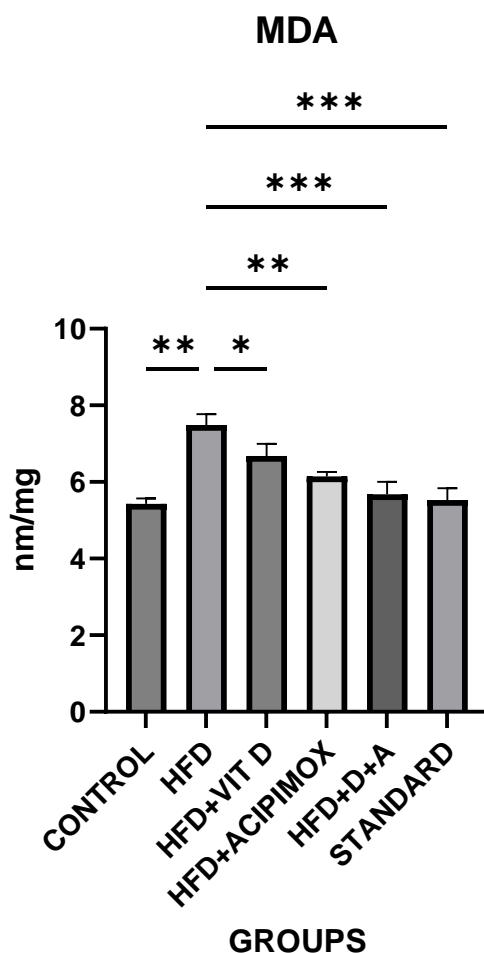
GSH

Figure 3.Data represent as mean ± SD; Effect of vitamin D and Acipimox on oxidative parameters (N=6 animals/group) against high fat diet induce diabetes on wistar rats. Data analyses was performed using one way ANOVA. *P<0.05 .,P<0.001,. control vs induced and induced vs treatment group.**

Thiobarbituric acid reactive substances (TBARS) measurement: The TBARS levels measured as an index of malondialdehyde (MDA) production were determined by the method of Uchiyama and Mihara(8). MDA, an end product of lipid peroxidation reacts with thiobarbituric acid to form a red coloured complex. The measurement of MDA levels by thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. Briefly, 1 g of the liver and

kidney samples were homogenised in 4 ml of 1.15% ice cold KCI using a Polytrin homogeniser (Kinematica GmbH, Lucerne, Switzerland) to form a 25% (w/v) homogenate. To 0.1 ml of 25% homogenate, 0.2 ml of 8.1% dodecyl sodium sulphate salt (SDS), 1.5 ml of 1% phosphoric acid, 0.2ml of distilled water and 1.0 ml of 0.6% 2-thiobarbituric acid were added. The mixture was heated in a boiling water bath for 45 minutes. Subsequently, the heated mixture was cooled in a ice bath, followed by an addition of 4.0 ml of n-butanol to extract the cold thiobarbituric acid reactants. The optical density of the n-butanol layer was determined at 353 nm after centrifugation at 1,000 g for five minutes and expressed as nmol MDA/25 mg wet weight.

Figure 4.Data represent as mean ± SD; Effect of vitamin D and Acipimox on oxidative parameters (N=6 animals/group) against high fat diet induce diabetes on wistar rats. Data analyses was performed using one way ANOVA. *P<0.05 .,P<0.001,. control vs induced and induced vs treatment group.**

RESULT: The effect of Vitamin D and Acipimox combination on free radical production, the activity of SOD, CAT, GSH and MDA were measured. They presented

significant increases in Acipimox and Vitamin treatment when compared with diabetic control rats. The effect of acipimox and vitamin D Combination was more prominent compared with standard

DISCUSSION: Hyperglycemia is a feature of diabetes mellitus, the most common metabolic ailment in the world. This condition is also characterised by changes in the intermediate metabolism of carbohydrates, proteins, and lipids. Reactive oxygen species are important in the aetiology, pathophysiology, and consequences of diabetes mellitus. In both insulin-dependent and non-insulin-dependent diabetes mellitus, lipid peroxidation. Antioxidant-based treatment has the potential to reduce the difficulties associated with diabetes mellitus by working to counteract the detrimental effects of oxidative stress. The goal of the current study was to assess the hypoglycemic and antioxidant status of Acipimox and Vitamin D on diabetes developed from a high-fat diet.

CONCLUSION: It suggests that administration of Acipimox and Vitamin D may be helpful in the prevention of diabetes and its complications associated with oxidative stress. Our results, therefore, suggest that the Acipimox and vitamin D could be used as a safe alternative antihyperglycemic drug for diabetic patients

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