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Studies on Anti-inflammatory and Anti-diabetic Potential of Andrographolide: Evidence from an *In vitro*, *In silico* and *In vivo* Study

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Abstract

Insulin function and sensitivity are compromised in type 2 diabetes (T2DM) due to various factors causing cellular stress and inflammation. With the increasing recognition of inflammation's role in both T1DM and T2DM, anti-inflammatory strategies are gaining importance in disease management. This study investigates the relationship between andrographolide and the enzymes α-glucosidase and α-amylase to elucidate its antidiabetic benefits. The study also evaluates andrographolide's ability to inhibit protein denaturation and examines its effects on the liver of T2DM rats through histological analysis. Methods included in vitro antidiabetic and anti-inflammatory activity assessments using α-glucosidase, α-amylase, and protein denaturation inhibition methods. Histopathological analysis of liver tissue from streptozotocin (STZ) and high-fat diet (HFD)-induced type-2 diabetic rats was conducted. In silico docking analysis was performed to confirm the binding affinity of andrographolide with pro-inflammatory signaling molecules. Data were analyzed using one-way ANOVA. Results indicated that molecular docking showed a good binding affinity with selected protein targets, attesting to andrographolide's powerful anti-inflammatory and antidiabetic effects. Histological analysis demonstrated that andrographolide could restore the hepatic architecture of diabetic livers. The in silico study further demonstrated high binding affinity against protein targets related to inflammatory and insulin signaling pathways. In conclusion, andrographolide may provide a promising basis for developing novel treatments and identifying critically needed pharmaceutical targets to address inflammation-related clinical problems in diabetes.

Keywords: *Andrographolide, Alpha-amylase, Alpha-glucosidase, Health and Well-being, Liver, Molecular Docking, Novel Methods, Protein Denaturation.*

Introduction

Diabetes poses a complex metabolic issue that disturbs the body's management of glucose levels. Insufficient pancreatic synthesis of insulin or the body's incapacity to properly use the insulin that is generated are the two main causes of this illness. Over 693 million instances of diabetes mellitus are expected worldwide by 2045, based on the International Diabetes Federation (IDF) [1].

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Economic growth and urbanization, which have drastically changed people's lifestyles by increasing obesity and decreasing physical exercise, are strongly linked to this rising prevalence.

A plethora of hypotheses and concepts have been put forward and proposed in an attempt to clarify the fundamental processes that give rise to diabetes. From a widely embraced perspective, inflammation characterizes a pivotal role in triggering the onset of diabetes. When pancreatic beta cells die, the pancreas cannot produce enough insulin, which leads to type 1 diabetes (T1DM). Multiple inflammatory agents contribute to this cell demise, including T-cell effectors targeting various beta-cell autoantigens and associated peptide epitopes. As the disease progresses, immune B cells undergo specific alterations, and macrophages contribute to inflammation within the islets through the output of reactive oxygen species, which directly harm beta cells. Furthermore, this pathogenic process may potentially involve natural killer T cells and dendritic cells [2].

In type 2 diabetes (T2DM), various factors leading to cellular stress can initiate inflammation, resulting in impaired insulin function and reduced insulin sensitivity. These factors encompass endoplasmic reticulum stress, oxidative stress, glucotoxicity, excessive lipid accumulation (lipotoxicity), ectopic fat deposits in organs like muscles, pancreas, and liver, as well as the buildup of amyloid proteins in the pancreas [3].

Employing anti-inflammatory techniques to improve both disease management and therapy has gained more traction as the importance of inflammation in T1DM and T2DM is becoming more widely acknowledged. Clinical trials employing anti-inflammatory therapies for both forms of diabetes have produced encouraging preliminary findings. These treatments include IL-1 antagonists and monoclonal antibodies, IKKbeta- NFkappaB inhibitors, and tumor necrosis factor (TNF) inhibitors. These treatments highlight the important role that inflammation plays in these illnesses. In managing inflammation and insulin resistance in T2DM, several anti-inflammatory medications, like thiazolidinediones and metformin are commonly administered [3]. However, their long-term use is significantly hampered by the adverse effects they can induce. To tackle this challenging situation, researchers and healthcare professionals are presently exploring the utilization of natural remedies to mitigate insulin resistance and inflammatory issues.

The 'King of Bitter,' scientifically named *Andrographis paniculata,* is a herbaceous plant of the *Acanthacea*e family. It is widely recognized and utilized globally. In traditional herbal medicine, this plant is used in Bangladesh, India, Malaysia, Hong Kong, China, and Bangladesh to treat ailments like laryngitis, fever, diarrhoea, and colds. Furthermore, it has a long track record of use in ethnobotany to cure fever, malaria, diarrhoea, diabetes, snake and insect bites, and other ailments [4]. The most often utilized parts of *A. paniculata* are its aerial parts, which are rich in flavonoids, flavonoid glycosides, glycosides, lactones, diterpenoids, and diterpenes. This plant has been associated with a wide spectrum of pharmacological benefits, including anticancer properties, antidiarrheal effects, anti-hepatitis activity, anti-HIV potential, lowering blood sugar effects, anti-inflammatory properties, antimicrobial activity, antimalarial capabilities, antioxidant activity, cardiovascular benefits, cytotoxic effects, hepatoprotective qualities, immunostimulatory properties, and potential assistance in managing sexual dysfunction. Many researchers are interested in confirming the efficiency of *A. paniculata* and investigating the mechanisms underlying its action due to its anti-diabetic qualities. *A. paniculate* aqueous extract has been demonstrated to enhance

glucose tolerance in mice, while an ethanol-based extract of the plant has demonstrated anti-diabetic effects in rats with diabetes induced by streptozotocin (STZ) [5]. Prior research indicates that ethanol extracts have the potential to efficiently reduce blood glucose levels in diabetic rats incited by STZ [6].

The generation of free radicals, as evidenced by numerous studies, significantly contributes to the initiation of inflammation. This shows that antioxidant supplementation may offer some protection against inflammatory diseases. The antioxidant qualities of the *A. paniculata* extract treatment account for the suppression of inflammation, and this enhances the plant's therapeutic potential [5]. These properties can be due to the existence of strong phytoconstituents present in this medicinal plant.

Andrographolide, one of the primary active components of *A. paniculata,* has a variety of biological effects, including anti-platelet aggregation, hepatoprotective, anti-allergic, anti-bacterial, anti-hyperglycemic, anti-malarial and anti-inflammatory effects. In addition to these actions, it has been shown that different extracts of *A. paniculata* can lower blood glucose levels in rats that have diabetes caused by streptozotocin. As a bipolar molecule, andrographolide can respond biologically in a variety of ways by interacting with a wide range of intra- and intercellular components in biological systems. According to a recent study, andrographolide and *A. paniculata* polysaccharides together can speed up the healing process for diabetic nephropathy [6]. This plant's phytoconstituents cause a number of pro-inflammatory genes to express less in different cell types, including leukocytes, synoviocytes, endothelial cells, and colorectal cancer cells [7].

The present research is centered on investigating how andrographolide exerts its anti-diabetic effects through its interaction with α-amylase and α-glucosidase enzymes. Further, it seeks to understand its anti-inflammatory properties by assessing its ability to inhibit protein denaturation. Additionally, the study intends to investigate the potential of andrographolide on the livers of rats with T2DM by histological examination. The *in silico* analysis is done to know the binding affinity of the andrographolide against the proposed targets.

Materials and Methods

Sigma Aldrich, based in St. Louis, USA, catered to the chemicals, andrographolide, and reagents for this work.

Assay of α-Amylase Inhibitory Activity

Test tube contents included andrographolide (20 µg/mL in DMSO), 480 µL of distilled H_2O, and phosphate buffer (20 mM) comprising 6.7 mM sodium chloride. Three minutes later, about 600 µL was taken out and put into several test tubes along with 300 µL of DNSA. The test tubes were immersed in a hot water bath sustained at a temperature range of 85 to 90 °C for fifteen minutes. Using a microwell plate reader, the absorbance at 540 nm was determined after the reaction liquid in each tube. Test incubations were set up to examine the concentration-dependent inhibition of andrographolide at 0.1 to 0.5 mg/mL. The maltose standard calibration curve equation, which is 0-0.1% w/v maltose, was used to determine the released maltose percentage (w/v) [8]. The inhibition level (%) was calculated using the given formula:

$$\%\ \text{reaction} = \frac{\text{Mean maltose in sample}}{\text{Mean maltose in control}} \times 100$$

Assay of α-glucosidase Inhibitory Activity

During the experiment, different doses of andrographolide were incubated for five minutes with a sample containing crude α-glucosidase protein (0.5 mg). Subsequently, a final reaction mixture was made up of phosphate buffer (0.1 M) as well as maltose

and sucrose substrates and incubated separately for 20 and 30 minutes at 37°C.1.0 mL of Tris base was added to stop the reaction, and the glucose oxidase technique was used to measure the amount of α-glucosidase activity based on the glucose liberated from maltose and sucrose [9].

Protein Denaturation Assay

After being incubated for 15 minutes at 37 °C, the reaction mixture containing 0.1–0.5 mg of andrographolide, 4.78 mL of phosphate-buffered saline (pH 6.4), and 1% bovine albumin was exposed to 70 °C for five minutes). The UV/VIS spectrometer was used to measure the turbidity at 660 nm. The control was the phosphate buffer solution [10].

% Inhibition of Denaturation = 100 × (1 − A2/A1)

where,

Absorption of the control sample- A1

Absorption of the test sample- A2

In vivo Study

T2DM Induction

In this investigation, rodents were given a high-fat diet (HFD) for 28 days. Three percent coconut oil, one percent cholic acid, three percent cholesterol, and sixty-six percent traditional rat meal made up the HFD. The rats were given an intraperitoneal injection of 35 mg/kg of streptozotocin (STZ) following 28 days of the high-fat diet [11]. Animals that had FBG levels more than 120 mg/dl were chosen for research inclusion two days after STZ was administered. These rats were divided into three groups, each consisting of five rats, with the selection process being random.

Experimental Design

Group 1 represents the control group, Group 2 consists of rats with HFD-STZ induced T2DM rats, and Group 3 comprises of HFD-STZ induced T2DM rats with oral administration of Andrographolide (1.5 mg/kg body weight twice daily for 21 days) [12].

On the last day of the experiment, the rats were given a dosage of thiopental equivalent to 40 milligrams per kilogram of their body weight to induce sleep. Once sedated, blood samples were collected via cardiac puncture. After the blood was drawn, sera were separated and the samples were kept cold (-80°C) for further examination. The blood was extracted by injecting an isotonic sodium chloride about 20 mL into the left ventricle. The livers of the rats were harvested and preserved in ten percent neutral buffered formalin. The liver samples were then cut into slices, and dyed with hematoxylin and eosin for microscopic analysis. They were then photographed at a magnification of ×100 for further investigation and research.

Histopathological Investigation on Liver

For this study, adult male Wistar albino rats aged between 150 to 180 days were selected. The conventional environmental conditions for these rats were maintained at 21 ± 2°C with a consistent temperature and specific humidity. The research protocol adhered to the procedures and approvals of the Institutional Animal Ethics Committee. Standard pellets were fed to the rats, and they were allowed unlimited access to water. The Central Animal House at Saveetha Dental College and Hospital in Chennai, Tamil Nadu, provided the study's facility.

In silico Molecular Docking Analysis of Andrographolide

Preparation of Protein

The three-dimensional crystal structures of the following proteins have been obtained in PDB (Protein Data Bank) format from the RCSB Protein Data Bank:

Interleukin-1 beta (IL-1 beta) with PDB ID: 9ILB.

Human mammalian target of rapamycin (mTOR) with PDB ID: 4DRI.

Tumor Necrosis Factor (TNF) with PDB ID: 1TNF.

Nuclear Factor kappa B (NF-κB) with PDB ID: 4G3D.

Preparation of Ligand

The structure of Andrographolide was obtained from the PubChem database.

Molecular Docking

The Ligand Fit module in Discovery Studio was utilized to conduct experiments related to molecular docking. During docking, ten different stances were created. Dock score values acquired following energy minimization were used to choose the best poses [13]. The best orientation of a molecule within the active site as well as clever minimization were employed in this. Based on their interaction of H-bond with the receptor and a consensus scoring system, the active compounds were selected.

Statistical Analysis

One-way ANOVA analysis was performed on the experimental data to determine the statistical significance between the different concentrations that were used. Significance was determined at the $p<0.05$ level, indicating that differences with a p-value less than 0.05 were considered statistically significant.

Results

Effect of Andrographolide on Alpha-amylase Inhibitory Activity

The findings of the investigation demonstrated a rise in the percentage of alpha-amylase activity inhibition that was dose-dependent for both andrographolide and acarbose, the usual medication, at concentrations between 100 and 500µg/ml (Figure 1). The highest anti-diabetic efficacy for both andrographolide and the standard drug was observed at the concentration of 500 µg/ml. Specifically, the inhibition percentages for andrographolide at concentrations of 100 µg to 500 µg were 15%, 20%, 40%, 80%, and 85%, respectively. These values were significantly comparable to those of Acarbose. Table 1 provides a detailed comparison of the inhibition percentages in alpha-amylase activity between andrographolide and acarbose.

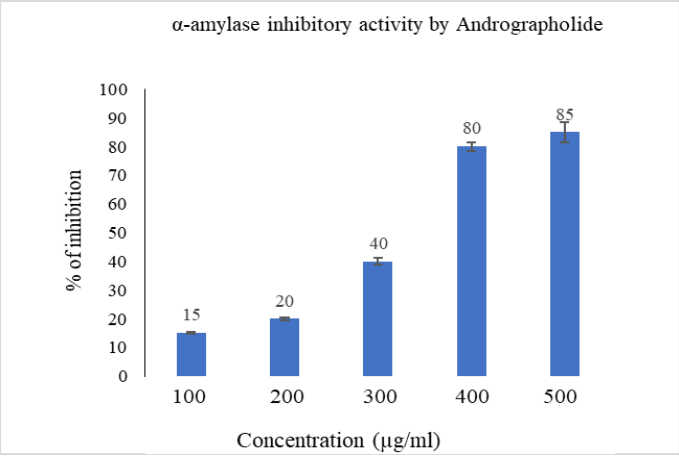

Figure 1. Effect of *Andrographolide* on α- amylase Inhibitory Activity.

Table 1. α- amylase Inhibition Percentage of Andrographolide and the Standard Drug- Acarbose

S.No	Andrographolide concentration (in µg/ml)	Extract % inhibition	Standard % of inhibition
1	100	15	20
2	200	20	40
3	300	40	60

4	400	80	80
5	500	85	90

Effect of Andrographolide on Alpha-glucosidase Inhibitory Activity

The study's results demonstrated a dose-dependent inhibition of α-glucosidase activity for both andrographolide and the standard drug within the concentration range of 100 to 500 µg/ml, as illustrated in Figure 2. The highest anti-diabetic activity for both andrographolide and the standard drug was observed at the concentration of 500µg/ml. In particular, the percentage inhibition of α-glucosidase activity by andrographolide was 10%, 14%, 30%, 40%, and 70% at concentrations of 100 to 500µg. These values were compared to the inhibition percentages of acarbose at different concentrations, as detailed in Table 2.

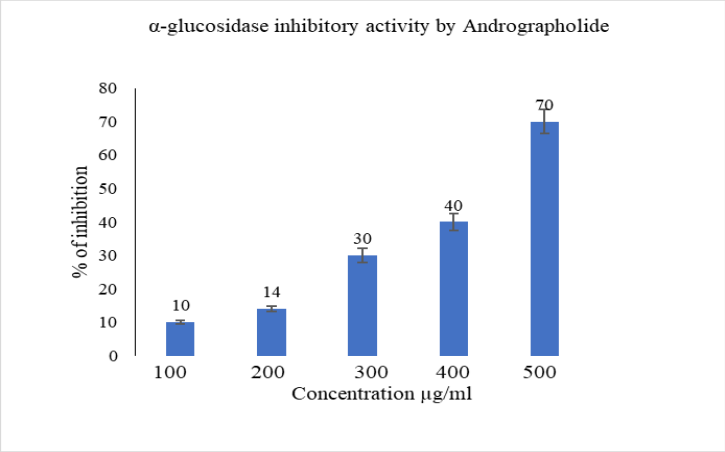

Figure 2. Effect of *Andrographolide* on α- glucosidase Inhibitory Activity.

Table 2. α- glucosidase Inhibition Percentage of Andrographolide and the Standard Drug- Acarbose.

S.No	Andrographolide concentration (in µg/ml)	Extract % inhibition	Standard % of inhibition
1	100	10	15
2	200	14	25
3	300	30	35
4	400	40	50
5	500	70	80

Effect of Andrographolide on Protein Denaturation Assay

Andrographolide exhibited a dose-dependent inhibition of protein denaturation, indicating its potential anti-inflammatory properties, as depicted in Figure 3. The percentage inhibition increased with higher concentrations, showing values of 10%, 23%, 36%, 48%, and 63% at concentrations of 100 µg to 500 µg. In contrast, acarbose, the conventional medication, likewise showed suppression of protein denaturation at dosages ranging from 100 µg to 500 µg, with percentage inhibition values of 23%, 39.4%, 43%, 54%, and 70%. It is noteworthy that Andrographolide displayed an increasing trend in the inhibition of protein denaturation, particularly between concentrations of 300 µg and 500 µg (Table 3).

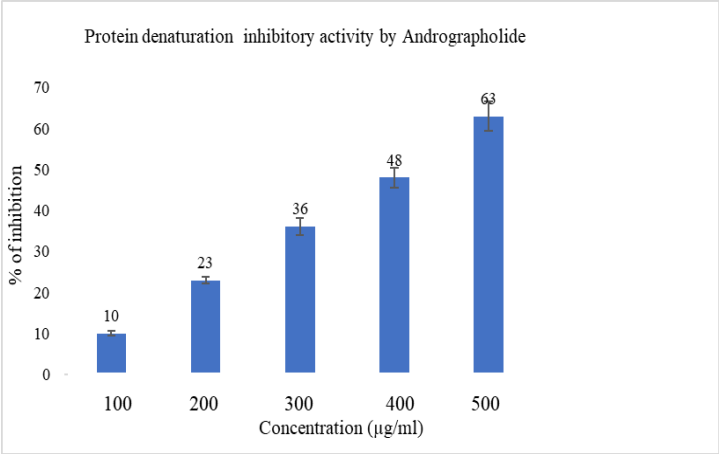

Figure 3. Effect of *Andrographolide* on Protein Denaturation Inhibitory Activity.

Table 3. Protein Denaturation Inhibition Percentage of Andrographolide.

S.no	Andrographolide concentration (in µg/ml)	Extract % inhibition	Standard % of inhibition
1	100	10	23
2	200	23	39.4
3	300	36	43
4	400	48	54
5	500	63	70

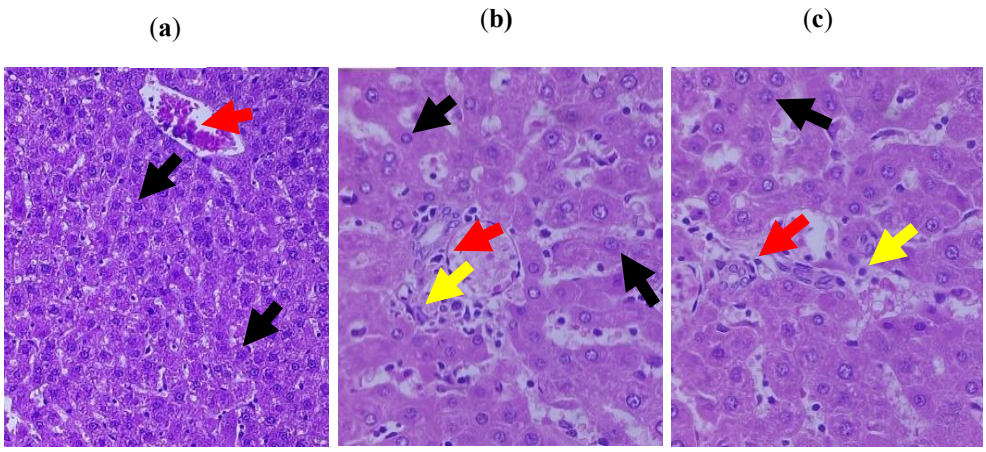

Figure 4. Histopathology of Liver Tissue Illustrating Andrographolide's Effects.

Effect of Andrographolide in the Histopathology of Live Tissues

In order to assess histological changes associated with diabetes and the potential restoration through Andrographolide treatment, histological examination using H&E staining was performed (as shown in Figure 4). In addition to displaying a clear and identifiable liver lobular structure, the liver histology of the normal group also showed hepatocytes organized correctly surrounding the central vein. However, there were prominent and widespread lipid vacuoles associated with a substantial buildup of fat

deposits in the liver of Group 2, which included rats with T2DM. These findings suggested severe micro-vesicular fatty changes and histological abnormalities. The histological analysis revealed that Andrographolide intervention in Group 3 led to a substantial reduction in these histological abnormalities. This suggests that Andrographolide treatment had a profound restorative effect on the liver tissues, nearly bringing them back to a normal, healthy state.

Molecular Docking

Protein-ligand interactions were assessed using molecular docking techniques. An overview of the number of hydrogen bonds created, the relevant amino acid associations, and its concerned score is provided in a table that summarizes the results of these interactions. Positive binding energies between the ligand and receptor protein indicate the presence of a strong, beneficial interaction. Table 4 provides information on the binding energy and interactions between andrographolide and the protein targets and a visual representation of these interactions can be found in Figure 5 & Table 4.

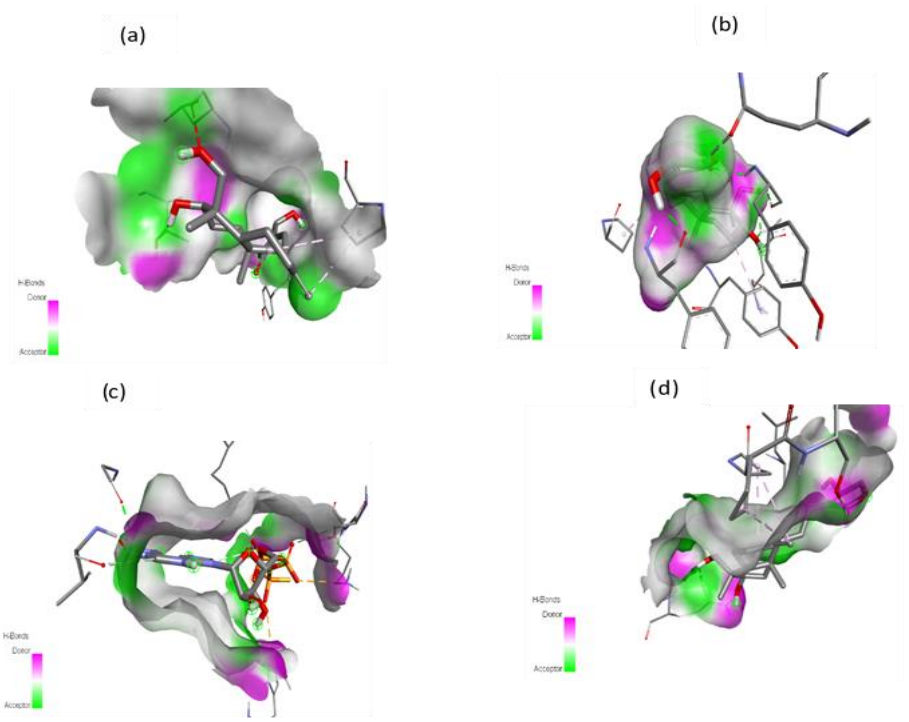

Figure 5. Molecular Interaction of Andrographolide with (a) IL-1β ; (b) TNF-α; (c) m TOR; (d) NFκB

Table 4. Molecular Interaction Results of Andrographolide with Selected Target Proteins.

Protein name	**Binding Energy Kcal/mol**
TNF-α	-4.8
IL-1BETA	-6.0
NFkB	-5.9
mTOR	-6.4

Discussion

Obesity can raise the risk of T2DM by causing insulin resistance. Adipocytes that reside in adipose tissue and hypertrophied immune cells define obesity, as a proinflammatory state marked by elevated circulation levels of proinflammatory cytokines [13]. The specialized macrophages of the liver, known as Kupffer cells, are responsible for producing chemokines and cytokines. Pro-inflammatory chemicals, which include surplus FFA and proinflammatory cytokines, draw in more macrophages and other immune cells when they are introduced or encountered locally. Elevated reactive oxygen species (ROS) also decrease insulin receptor substrate recruitment, which obstructs the downstream PI3K-AKT cascade from activating. Pro-inflammatory cytokines can cause peripheral target organs to produce acute-phase proteins, insulin resistance, and pancreatic β-cell death. These gradually become pro-inflammatory instead of anti-inflammatory, and it is thought that this association with the cells of peripheral target organs causes insulin resistance [14].

An increasing variety of plants from various countries are recognized to have anti-diabetic qualities, and herbal therapies are widely used as supplements to modern medications to treat diabetes and its aftereffects. More than 1200 plants have hypoglycemia potential, according to ethnopharmacological studies, and in ancient Indian literature, more than 800 herbs with antidiabetic properties are listed [15]. Two enzymes called α-amylase and α-glucosidase hydrolyze carbohydrates, which is the main cause of postprandial hyperglycemia. α-amylase starts the degradation of carbohydrates by releasing 1, 4-glycosidic linkages between polysaccharides, which results in the production of disaccharides, like starch and glycogen. α-glucosidase then converts these disaccharides into monosaccharides, causing hyperglycemia after meals [16]. In order to manage hyperglycemia, inhibitors of α-amylase and α-glucosidase reduce the postprandial plasma glucose level by delaying the digestion of carbohydrates. Alpha-amylase inhibitors are essential to assist in lowering the heightened glucose levels that can occur following a meal. This effect is particularly important for individuals with diabetes, as insufficient insulin levels hinder the rapid removal of excess glucose from the bloodstream. Therefore, to maintain control over their blood glucose levels, diabetics typically have low alpha-amylase levels. In this study, the alpha-amylase concentration ranged from 100 to 500µg/ml, demonstrating a dose-related higher inhibition % concerning andrographolide as well as acarbose. At 400µg/ml and 500µg/ml, the inhibitory concentrations were almost the same as those of the reference drug. In the alpha-amylase inhibition experiment, the methanolic extract of *Flavoparmelia caperata* exhibited the highest degree of inhibition (49% at 15 mg/ml), followed by *Physcia aipolia* (46% inhibition), according to related research by Shivanna et al. (2015) [17]. According to Subramanian et al. (2008), andrographolide produced a maximal inhibition of 54.8% at 10 mg/ml in the alpha-amylase inhibition experiment [18].

T2DM is treated with oral anti-diabetic medications called alpha-glucosidase inhibitors and they work by inhibiting the breakdown of starches and other carbohydrates. Normally, simple sugars made from carbohydrates can be taken through the intestines [19]. In the small intestine, complex carbohydrates are hydrolyzed to glucose and other monosaccharides by intestinal alpha glucosidases. Carbohydrate digestion is slowed down in part by inhibiting these enzyme systems [20]. Less glucose is absorbed because carbs cannot be transformed into glucose molecules. In the near run, diabetics with high blood glucose levels benefit from these enzyme inhibitor drug regimens. Consequences of using synthetic enzyme

inhibitors include abdominal bloating, diarrhoea, and flatulence in the gastrointestinal tract [21]. It is therefore possible to efficiently treat post-prandial hyperglycemia using natural alpha-amylase and glucosidase inhibitors that come from dietary plants, with little to no negative side effects. In an independent investigation, the alpha-glucosidase assay yielded 129.85±10.29, 76.90±9.55, 140.01±10.08, and 96.56±12.93 µg/ml [1C50 values] for the plant extracts of *Artocarpus altilis*, *Artocarpus heterophyllus*, *Cinnamomum zeylanicum,* and Piper betel, respectively [22]. Additionally, all four plant extracts exhibited a rise in inhibitory action against alpha-glucosidase that was dependent on the dosage. The alpha-glucosidase assay in our investigation also showed a dose-dependent inhibition, suggesting that it might be helpful in the treatment of postprandial hyperglycemia.

According to certain theories, inflammation is a complex physiopathological reaction to various stimuli. Mediators include prostaglandins, cytokines, ROS, neutrophil-derived free radicals, and NO are associated with the process. When these mediators are produced in excess, they damage macromolecules and cause lipid peroxidation of the membrane, which results in tissue damage. Thus, tissue damage serves as a key marker for the pathophysiology of numerous inflammatory illnesses. Hence, antioxidants and radical scavengers can reduce inflammation by neutralizing free radicals, which are crucial mediators that start or maintain inflammatory processes [23]. Denaturation of proteins, which outcomes in the loss of their biological function, is one cause of inflammation. Therefore, it is possible to reduce inflammatory activity by inhibiting protein denaturation [24]. The gradient inhibition rate was noted in the present investigation, with the maximal concentration being 500µg/ml. Similarly, Ficus racemosa's anti-inflammatory properties were assessed using the denaturation of egg albumin method, according to Dharmadeva et al. (2018). At a dosage of 1000 µg/ml, the maximum inhibition rate was noted in extracts made with both hot and cold water. At 0.01 µg/ml and 0.1 µg/ml doses, hot water extractions showed noticeably more inhibition than cold water extractions [25]. In another study, it was discovered that at a concentration of 500 µg/ml, *A. paniculata* demonstrated an impressive inhibition rate of 90.1%. In comparison, Diclofenac, which is a widely used standard anti-inflammatory drug, exhibited a slightly lower maximum inhibition of 82.6%. This result raises the possibility that *A. paniculata* has a strong anti-inflammatory activity that, in certain experimental settings, may exceed Diclofenac [26]. These previous works support our findings on the anti-inflammatory potential of andrographolide.

The primary contributors to hepatocyte fatty degeneration are insufficient insulin and abnormalities in the pathway of mitochondrial fatty acid β-oxidation. Fatty acids are converted into many triglyceride droplets in the hepatocytes as a consequence. In inflammatory situations like obesity and T2DM, kupffer cells become activated and release a large amount of inflammatory chemokines and cytokines [27]. As a result, our study, which was similar to Roy et al.'s work, revealed significant findings in the histological examination of liver slices from rats with type 2 diabetes mellitus (T2DM). Hepatocyte injury, cellular inflammation, vascular congestion and fatty deposit formation in hepatic tissue were among these observations [28]. A similar investigation was carried out by Motshakeri et al. [29]. In this study, medicament with andrographolide progressively restored hepatocyte architecture, which reduced cellular inflammation. These demonstrate andrographolide's capacity for hepatoprotection as well as its function in hepatic insulin signaling.

The binding affinity of molecules in an *in silico* analysis is affected by non-covalent intermolecular interactions such as hydrophobic, Van der Waal, electrostatic, and hydrogen bonding interactions. An extra molecule's presence may also have an impact on a ligand's affinity for binding to a receptor's active site. The degree of binding affinity and its free energy that indicate the intensity of the interaction between a protein and a ligand (e.g., andrographolide) offers important information about the possible mechanisms of action of the ligand through different routes. Furthermore, a key element affecting the emergence of pharmacological action is the structural configuration of the ligand-receptor complex. Of the receptors examined in this work, andrographolide showed a substantial binding affinity concerning binding energy as well as hydrogen and hydrophobic interactions. This finding holds promise for researchers seeking new anti-diabetic drugs, as it suggests that andrographolide has a robust interaction with these proteins. It is crucial to remember that, even though these computational studies are instructive, more experimental work is required to confirm the precise interaction between the bioactive components of andrographolide and the aforementioned proteins. This would be a crucial step in advancing our understanding and potential therapeutic applications of andrographolide in the context of diabetes.

Conclusion

These findings validate that andrographolide strongly possesses anti-inflammatory and anti-diabetic effects. The histopathological investigation also demonstrated that this promising phytoconstituent can reinstate the hepatic microstructure of diabetic livers. Furthermore, the *in silico* investigation proved the strong binding affinity against the protein targets involved in the insulin and inflammatory signaling pathways. Consequently, andrographolide could serve as a promising foundation for initiating research into new therapeutic possibilities and urgently required pharmacological targets to address the pathological conditions associated with inflammation in diabetes.

Conflict of Interest

The authors hereby declare that there is no conflict of interest.

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