Predictive Algorithm for evaluating the efficacy of mushroom blends for optimizing Type 2 diabetes intervention

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Abstract

Natural sources such as herbs, nutraceuticals, and medicinal mushrooms are increasingly used to manage diseases and promote wellness by balancing metabolic, immune, and oxidative stress. Type 2 diabetes (T2D), characterized by metabolic dysregulation, is a key area where these natural interventions show significant promise. In this study, a predictive algorithm was developed and applied to assess the therapeutic potential of three specific mushrooms—Pleurotus citrinopileatus, Pleurotus sajor-caju, and Pleurotus ostreatus—in managing T2D biomarkers. The algorithm was first validated against existing literature, accurately predicting the effects of these mushroom combinations on key biomarkers, including fasting glucose, HbA1c, insulin, HOMA-IR, triglycerides, LDL/HDL ratio, and total cholesterol, with a maximum fold change difference of 0.5 between expected and predicted values.The algorithm also identified an optimal dosage that maximized biomarker improvement while considering cost efficiency. Simulated outcomes for the optimal combination (C19), comprising 50% P. ostreatus, 25% P. sajor-caju, and 25% P. citrinopileatus by net weight , indicated a 40% reduction in glucose levels, a 17% decrease in insulin levels, a 45% drop in triglyceride levels, and a 13% reduction in cholesterol levels within one month in a diabetic population. The formulation consistently normalized biomarker levels within three months. A case study using the C19 combination as an intervention further validated the algorithm, highlighting its accuracy and reliability through a close correlation with actual outcomes. By integrating literature data, case study validation, and cost analysis, this approach offers a robust framework for designing effective and economical interventions for T2D.

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ABSTRACT

Natural sources such as herbs, nutraceuticals, and medicinal mushrooms are increasingly used to manage diseases and promote wellness by balancing metabolic, immune, and oxidative stress. Type 2 diabetes (T2D), characterized by metabolic dysregulation, is a key area where these natural interventions show significant promise. In this study, a predictive algorithm was developed and applied to assess the therapeutic potential of three specific mushrooms—*Pleurotus citrinopileatus*, *Pleurotus sajor-caju*, and *Pleurotus ostreatus*—in managing T2D biomarkers. The algorithm was first validated against existing literature, accurately predicting the effects of these mushroom combinations on key biomarkers, including fasting glucose, HbA1c, insulin, HOMA-IR, triglycerides, LDL/HDL ratio, and total cholesterol, with a maximum fold change difference of 0.5 between expected and predicted values.The algorithm also identified an optimal dosage that maximized biomarker improvement while considering cost efficiency. Simulated outcomes for the optimal combination (C19), comprising 50% *P. ostreatus*, 25% *P. sajor-caju*, and 25% *P. citrinopileatus* by net weight , indicated a 40% reduction in glucose levels, a 17% decrease in insulin levels, a 45% drop in triglyceride levels, and a 13% reduction in cholesterol levels within one month in a diabetic population. The formulation consistently normalized biomarker levels within three months. A case study using the C19 combination as an intervention further validated the algorithm, highlighting its accuracy and reliability through a close correlation with actual outcomes. By integrating literature data, case study validation, and cost analysis, this approach offers a robust framework for designing effective and economical interventions for T2D.

Introduction

Natural Sources are increasingly being used to manage disease and wellness. These sources from herbal, food constituents and nutraceuticals are used in balancing metabolic, immune and oxidative stress health. One of the diseases where the metabolic dysregulation results in type 2 diabetes, is an example where nutraceuticals and herbal formulations can offer benefit in managing the disease.

Of the natural sources available to treat diabetes, mushrooms have gained attention for their therapeutic benefits and have been valued in traditional medicine for centuries Their bioactive compounds can enhance insulin sensitivity and reduce blood sugar levels. Additionally, mushrooms are abundant in antioxidants, vitamins, and minerals, which can help alleviate complications associated with diabetes. Edible mushrooms have been employed to maintain health and promote longevity since ancient times (Das et al., 2019). Oyster mushrooms, in particular, are known for their

significant nutritional and medicinal benefits and are especially popular in countries like India, China, and Japan. These mushrooms are highly nutritious, being low in calories and fats while rich in essential fatty acids, proteins, vitamins, and minerals. They have demonstrated significant hypoglycaemic potential in animal models, both in acute and chronic settings. Additionally, oyster mushrooms are known for their antioxidant properties, which can help combat oxidative stress, a common issue in diabetes. Their high fiber content also aids in digestion and can contribute to better blood sugar control. Despite these benefits, further research is needed to fully understand and harness their therapeutic potential. In recent years, numerous animal studies have explored the impact of oyster mushrooms, either dried or as extracts, on blood lipid profiles. These studies have shown significant reductions in triacylglycerol (TG) concentrations, as well as total and LDL cholesterol levels, when using dried oyster mushrooms or their extracts (Schneider et al., 2011)The notable and popular oyster mushrooms include *Pleurotus sajor caju*, *Pleurotus ostreatus*, and *Pleurotus citrinopileatus*. *P.sajor caju*, cultivated globally, is rich in high-quality proteins and vitamins such as B1, B2, and C, while containing very little fat or starch. It is believed to reduce cholesterol levels in the blood and prevent hyperlipidemia due to its low-fat and high soluble fiber content (Miklos et al., 2011) *Pleurotus ostreatus*, known for containing the HMG-CoA-reductase inhibitor mevinolin (lovastatin), may contribute to a lipid-lowering effect, making it beneficial for managing cholesterol levels. P.citrinopileautus is a nutritious and flavourful edible mushroom, with recent research on animal models suggesting it may have physiological benefits such as antitumor, immune-enhancing, and antihyperglycemic effects (Shu et al., 2006a)

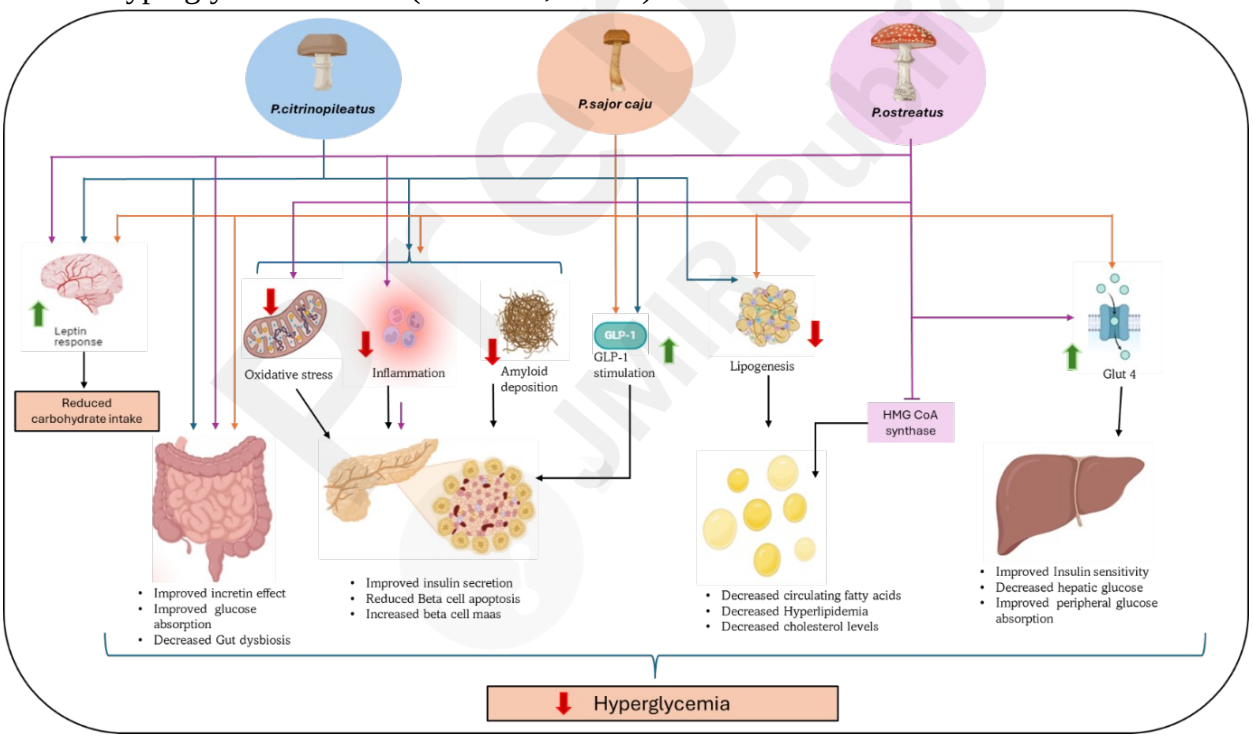


Figure 1. **Mechanism of action of mushrooms over various pathophysiology aspects of type 2 diabetes**.

In managing type 2 diabetes, the therapeutic potential of *P.citrinopileatus*, *P.sajor caju, and P.ostretus* is highlighted by their diverse mechanisms of action. *P.citrinopileatus* reduces dietary carbohydrate intake, improving the gradual intestinal absorption of glucose due to the presence of quercetin. It upregulates antioxidant pathways because of catechin, leading to the protection of pancreatic beta cells from apoptosis (Yin et al., 2019) .Additionally, it enhances leptin signaling, which improves feelings of satiety and aids in weight management, attributed to the presence of

compounds such as phenolic acids (including alpha-linolenic acid) (Kato et al., 2000). It also decreases mitochondrial dysfunction and reduces inflammation, which enhances insulin secretion and blood glucose control. Furthermore, the presence of vitamins like Riboflavin improves beta-cell function and upregulates GLUT4, facilitating glucose uptake in muscle and adipose tissues (Raman et al., 2020) *P. sajor caju* demonstrates comparable benefits through its ability to reduce mitochondrial dysfunction, mitigate inflammation, and enhance beta-cell function, harnessing the therapeutic potential of phenolic acids like ferulic and coumaric acid. Additionally, its abundance of flavonoids like myricetin aids in increasing GLP-1 secretion and reducing amyloid deposition. (Krümmel et al., 2022). These actions improve energy metabolism and help control blood sugar levels. Similarly, due to its content of beta-carotene (a carotenoid) and chrysin (a flavonoid), *P. ostreatus* aids diabetes management by improving leptin response, reducing mitochondrial dysfunction, and exerting anti-inflammatory effects(Farkhondeh et al., 2019; Golak-Siwulska et al., 2018). Due to its linolenic acid content, it enhances GLP-1 secretion, supports beta-cell function, and upregulates GLUT4, thereby improving glucose uptake and lowering blood glucose levels. Collectively, these mushrooms positively impact gut health, support pancreatic function, regulate fat metabolism, and improve liver function. By addressing these pathways, *P.citrinopileautus, P.sajor caju*, and *P.ostreatus* effectively manage hyperglycemia and reduce the pathophysiology of diabetes.

In this study, we aim to develop and apply a predictive algorithm to assess the impact of interventions using combinations of three mushrooms (*P.citrinopileautus, P.sajor caju, and P.ostreatus*) on Type 2 Diabetes (T2D) biomarkers. Using this algorithm, we plan to predict the levels of fasting glucose, HbA1c, insulin, HOMA-IR, triglycerides, LDL/HDL ratio, and total cholesterol following interventions with different combinations of the three mushrooms. Additionally, we will employ the algorithm to determine the optimal dosages of mushroom combinations that yield the most significant reductions in the targeted biomarkers while considering the cost of the formulations. By integrating literature-reported data, case study validation, and cost analysis, our approach aims to provide a comprehensive framework for designing effective and economical T2D interventions. This study underscores the potential of computational models in advancing diabetes treatment by accurately predicting the effects of mushroom-based interventions and optimizing their dosages for both efficacy and cost.

Methodology

Database Construction and Study Screening

The methodology employed in this study involves the construction of a database from public resources on mushrooms. In the screening process, research articles, meta-analyses, randomized controlled trials, and review articles on three mushrooms (*P. citrinopleatus, P. ostreatus, P. sajor caju)* were evaluated based on specific inclusion criteria. The studies selected involved participants with Type 2 Diabetes (T2D) or those exhibiting fasting glucose levels above 100 mg/dL and HbA1c levels exceeding 6.5%. Furthermore, the selected studies needed to report effects on one or more Type 2 Diabetes markers, such as fasting glucose, postprandial glucose, HbA1c, insulin levels, triglycerides, and HOMA-IR.

Algorithm explanation and Validation

Firstly, the database incorporates the rates of metabolite change due to mushroom consumption, which serves as a key element in our algorithm. This algorithm is designed to predict post-intervention concentrations based on user-defined doses, durations, and combinations of various

mushrooms using the rate of change of biomarker concentration from the database. To compute the normalized rate of change in biomarker levels or concentrations, the formula mentioned below was employed. This normalization ensures that the data remains consistent and interpretable across various different case studies, dosages and duration, facilitating meaningful comparisons.

$$\text{Normalized change} = \frac{(¿ - G0) - (Pt - P0)}{\left(\frac{D}{BW}\right) \times \left(\frac{t}{30}\right)} \quad \text{conc.(g dose. month /Kg BW)}^{-1}$$

The equation for normalized change serves as a method to quantify and compare changes in the concentrations of a biomarker before and after an intervention, while considering factors like dose, body weight, and duration. In this formula, Gt and G0 represent the concentration of the biomarker after and before intervention, respectively. Similarly, Pt and P0 denote the placebo concentrations of a biomarker after and before intervention. The ratio D/BW takes into account the dose relative to body weight, and t represents the elapsed time. The division by 30 to standardize the units as per month for duration. The equation computes the normalized change or rate by comparing the differences in biomarker concentrations, adjusting for factors like dose, body weight, and time. This normalization facilitates meaningful comparisons across diverse conditions or subjects. The rates we figure out become crucial for estimating final health marker levels, considering factors like weight and duration of intervention. The predicted health marker levels fall within a certain range, matching values found in studies after people take the supplements. This algorithm was validated using the collected knowledge base, by simulating the same intervention conditions and comparing the post intervention concentrations of biomarkers with the predicted levels. This approach, built on a deep understanding of existing data, offers a quick and effective alternative to traditional, time-consuming methods. The algorithm functions proficiently in predicting post-intervention values for both singular interventions and combinations involving nutraceuticals, showcasing a notably reduced deviation from the anticipated outcomes. Subsequently, the same algorithm is applied to optimize the dosage for the considered species of mushrooms when used in combination. However, the data used for these predictions is exclusively from randomized controlled trials (RCTs). There have been instances where RCTs for several supplements, especially those involving human subjects, have been limited, making the algorithm heavily dependent on data availability and quality of the data. Nonetheless, the algorithm performs proficiently in predicting post-intervention values for both single interventions and combinations involving supplements, demonstrating a notably reduced deviation from expected outcomes.

Analysis of Mushroom Effects on Biomarkers

Once the database was built according to the prescribed format, the data was analysed and the results reported in the literature for each mushroom and their effects on various biomarkers were examined. Specifically, the reduction in each biomarker was analysed for the average dose reported in the literature over a one-month period for an average 70 kg individual. This process involved determining the average doses by aggregating dosage information from the literature for each mushroom.

Formulation Development and Selection of optimal formulation

Using the results from the meta-analysis, we created various formulations by adjusting the dosages

of three mushrooms. Approximately nineteen formulations were developed and their impact on different metabolic markers was simulated using the algorithm on an average 70 kg individual with unhealthy biomarker levels for a duration of a month and ranked based on their performance. From these rankings, we selected the top combinations out of which one of them was selected for further analysis based on efficacy and cost.

Population analysis

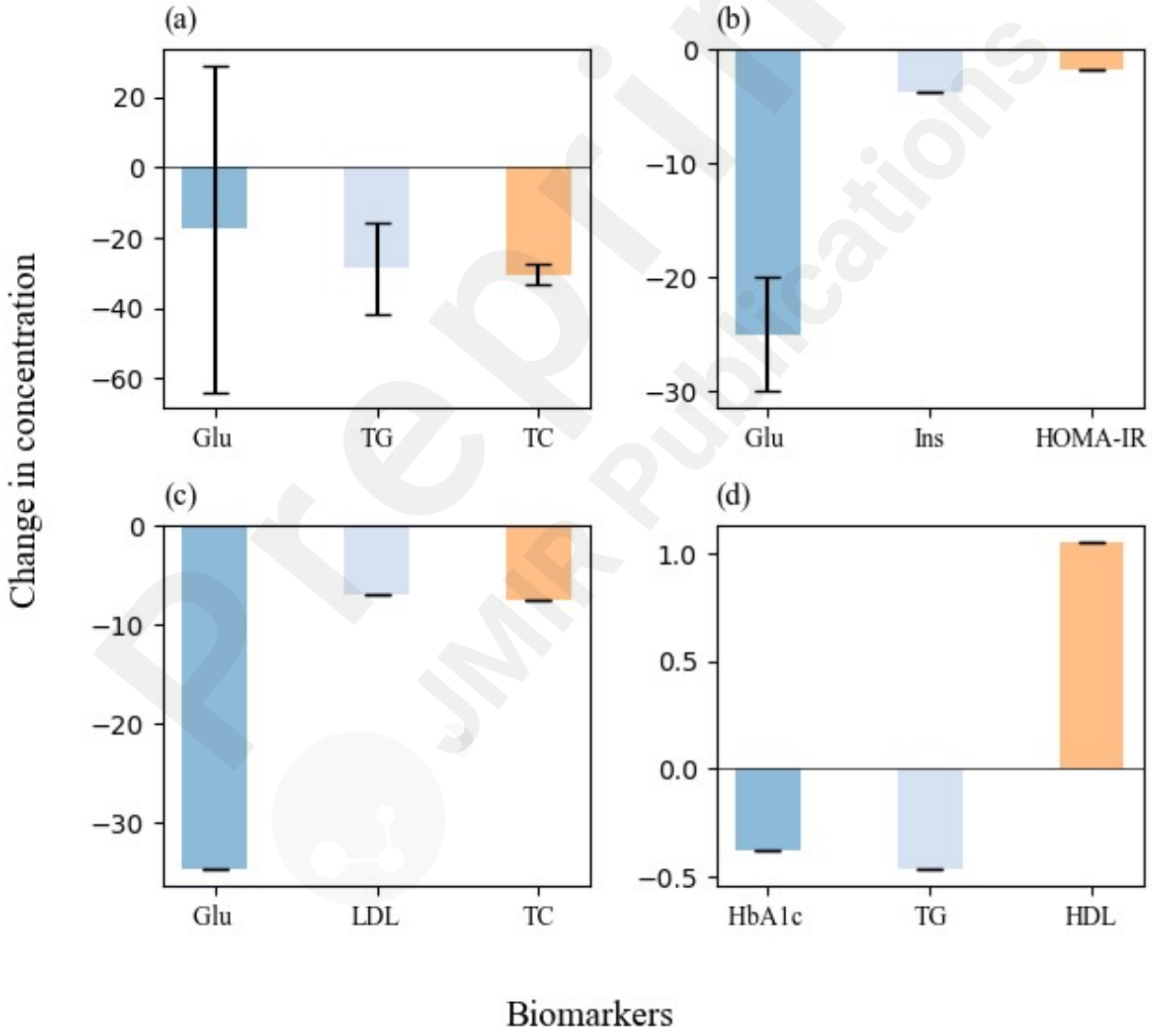
The data required for in silico population analysis was extracted from public repository of National Health and Nutrition Examination Survey (NHANES), program of Centre for Disease Control and Prevention (CDC) conducted across the United States. This data contains participants from various ethnicity living in the country and comprises individuals of different age and BMI. The raw data was processed to filter the diabetic individuals under the criteria of fasting blood sugar greater than 150 mg/dL. The biomarkers including lipids (triacylglycerols, LDL/ HDL ratio, total cholesterols), HbA1c, insulin, and HOMA-IR of the shortlisted candidates were also extracted for analysis. The post-intervention effects of the optimized dosage formulation were simulated on this filtered dataset using the algorithm, and subsequent changes in population biomarker levels were meticulously analysed to assess the efficacy of the intervention.

Results

Comparative Analysis of Effect of mushrooms across T2D Biomarker: A Meta-Analysis and Literature Benchmark

Figure 2.Meta analysis of Effects of Mushroom Consumption on Metabolic Markers. Each bar plot-(a)*P.citrinopileautus*,(b)*P.sajor caju*,(c and d)*P.ostreatus*- represents the change in the biomarker levels after consumption of the mushroom for the literature reported average dosage and duration. The abbreviations used are as follows: Glu-Fasting Glucose (mg/dL), TG-Triglycerides(mg/dL), TC-Total Cholesterol(mg/dL), Ins-Insulin(IU/L), HDL-High density lipoprotein(mg/dL), LDL-Low density lipoprotein(mg/dL).

The data from various case studies (RCTs) of three mushrooms on T2D study group was collected. There were limited RCTs for *P. citrinopileatus*; therefore, animal studies were considered and adjusted to be equivalent to human serum levels. The collected data was used to calculate the rate of change of biomarkers, as explained in the methodology section. These rates served as metrics to evaluate the effectiveness of each mushroom as an intervention to restore biomarkers to healthy levels. The collected data was used to simulate the impact of each mushroom on various biomarkers at the



average dose reported in the literature over a three-month period (Banukie N Jayasuriya et al., 2015; Choudhury et al., 2013; Kanagasabapathy et al., 2012; Sheng et al., 2019; Vishwakarma et al., 2023). The effects of *P. citrinopileatus, P. ostreatus, and P. sajor-caju* on various health markers are depicted in Figure 2.

The findings suggest that *P. citrinopileatus* can lower glucose levels by 20 mg/dL, triglycerides by

30 mg/dL, and total cholesterol by 35 mg/dL. *P. ostreatus* consistently shows beneficial effects on various markers, such as reducing glucose by 35 mg/dL, HbA1c by 0.5%, triglycerides by 0.5 mg/dL, LDL by 10 mg/dL, and total cholesterol by 10 mg/dL, while also raising HDL levels by 1 mg/dL, demonstrating its overall positive influence on health. Conversely, *P. sajor caju* significantly enhances insulin sensitivity, as reflected by a decrease in the HOMA-IR index by 2, insulin levels by 5 IU/L, and glucose by 25 mg/dL. Of the three, *P. ostreatus* is the most effective for glucose and HDL, *P. citrinopileatus* excels in managing triglycerides and total cholesterol, and *P. sajor caju* is the best for insulin and HOMA-IR.This meta-analysis highlights the potential health benefits of these specific mushroom species. After collecting and analysing data on the impact of each mushroom on various biomarkers, algorithm's predictive capability was validated using data from case studies. In silico simulations were employed for experimental design in each RCT, and the observed fold changes with respect to initial condition in biomarkers from these RCTs were compared with the fold changes predicted by the algorithm. Figure 3. illustrates the literature observed and algorithm predicted fold change plot for biomarkers, including glucose, triglycerides, total cholesterol, LDL, and HDL, after administering all three mushrooms as an intervention.

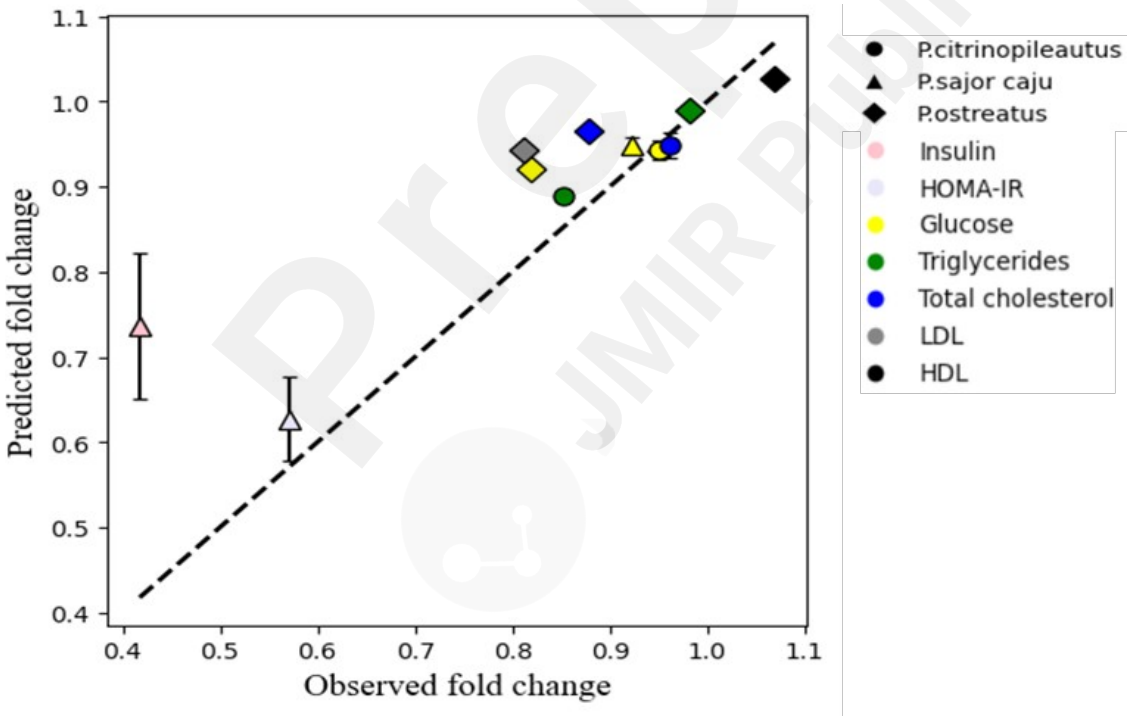


Figure 3. Predicted and Observed Fold Changes in Metabolic Biomarkers.The biomarkers are differentiated by color and mushrooms are denoted by shapes.

In the fig.3, most data points closely align with the diagonal line, indicating strong predictive accuracy by the algorithm. However, the insulin data point for *P. sajor caju* deviates by 0.3, revealing a slight underprediction tendency of the algorithm.

Formulation of optimal dose combinations for better efficacy in treating T2D

Table .1.Percentage Composition of Mushrooms in various combinations

Nineteen combinations were created by adjusting the dose percentages of each mushroom (Table 1), with the constraint of maintaining the total weight. These combinations were then used in simulations to assess their impact on various metabolic markers The effects of all 19 combinations on the biomarkers were simulated over the course of a month. The results have been visualized in terms of the percentage decrease for each biomarker using a bar plot (Fig.S1). To gain deeper insights into the most effective combinations for each biomarker, a performance cutoff was established. Subsequently, the combinations that outperformed the specified cutoffs were selected and analysed.

	Percentage concentration of mushrooms		
Combinations	*P.ostretus*	*P.sajor caju*	*P.citrinopileautus*
C1	12.5	50	12.5
C2	25	37.5	12.5
C3	37.5	25	12.5
C4	50	12.5	12.5
C5	12.5	62.5	12.5
C6	25	50	12.5
C7	37.5	37.5	12.5
C8	50	25	12.5
C9	62.5	12.5	12.5
C10	12.5	75	12.5
C11	25	62.5	12.5
C12	37.5	50	12.5
C13	50	37.5	12.5
C14	62.5	25	12.5
C15	75	12.5	12.5
C16	12.5	37.5	12.5
C17	25	25	12.5
C18	37.5	12.5	12.5
C19	50	25	25

Figure 4. Comparative Analysis of Blood Biomarkers across shortlisted formulations. Impact of formulations that showed more than 25% decrease in Glucose (a), more than 4% decrease in HbA1C (b) ,reduction of over 50% in insulin levels (c) , corresponding decrease of over 20% in the HOMA-IR (d) decrease of more than 30% in Triglycerides(e)

,reduction of 20% in total cholesterol (f) and more than 8 % decrease in LDL/HDL ratio(g) over a month.

Among the shortlisted combinations, C19 demonstrated the most robust overall impact, with reductions of 35% in glucose, 40% in triglycerides, and 27% in total cholesterol, making it the top performer across these biomarkers. This efficacy is likely due to the combined concentrations of *P.ostreatus* (50%) and *P.citrinopileatus* (25%) in the formulation. Additionally, C19 reduced HbA1c by 5%, placing fourth in this category, and improved the HDL/LDL ratio by 10%. In contrast, combination C15 showed the most significant effects on HbA1c and the HDL/LDL ratio, with reductions of 6.5% and 15%, respectively. This combination also ranked second in terms of glucose, triglycerides, and total cholesterol reductions, achieving decreases of 32%, 35%, and 22%. The potency of C15 is attributed to its composition, particularly the 50% concentration of *P. ostreatus* and 37.5% of *P. sajor caju*.

Combination C14 performed well, ranking third with a 31% decrease in glucose, 5.5% in HbA1c, and 12.5% in the LDL/HDL ratio. It also secured the fourth position in triglycerides and total cholesterol reductions, with decreases of 31% and 21%, respectively. Furthermore, C9 ranked as the second-best performer in improving the LDL/HDL ratio and HbA1c, with decreases of 12% and 5.5%. It came in third for reducing triglycerides and total cholesterol, showing decreases of 34% and 22%, respectively. The effectiveness of C9 in lipid management is likely due to the high concentration of *P. ostreatus* (62.5%). The C4 formulation demonstrated reasonable efficacy, particularly in reducing triglycerides and total cholesterol by 31.5% and 21.5%, placing it fifth in these metrics. It also ranked fourth in improving the LDL/HDL ratio (10%) but showed a lower impact on glucose and HbA1c reductions, with decreases of 27% and 4.5%, respectively, positioning it seventh overall. Finally, C10 emerged as the most effective formulation in reducing HOMA-IR, with a 40% decrease, a result attributed to the high concentration of *P. sajor caju* (75%) in the combination.

Among all the formulations investigated, the combinations that demonstrated a comprehensive impact across all biomarkers and had a reasonable manufacturing cost were C15, C4, C19, C14, and C10. To better understand how the selected formulations impact biomarkers and to identify the most effective formulation over extended period, the behaviour of these five combinations over a four-month period was assessed.

Effect of duration on the performance of selected formulations:

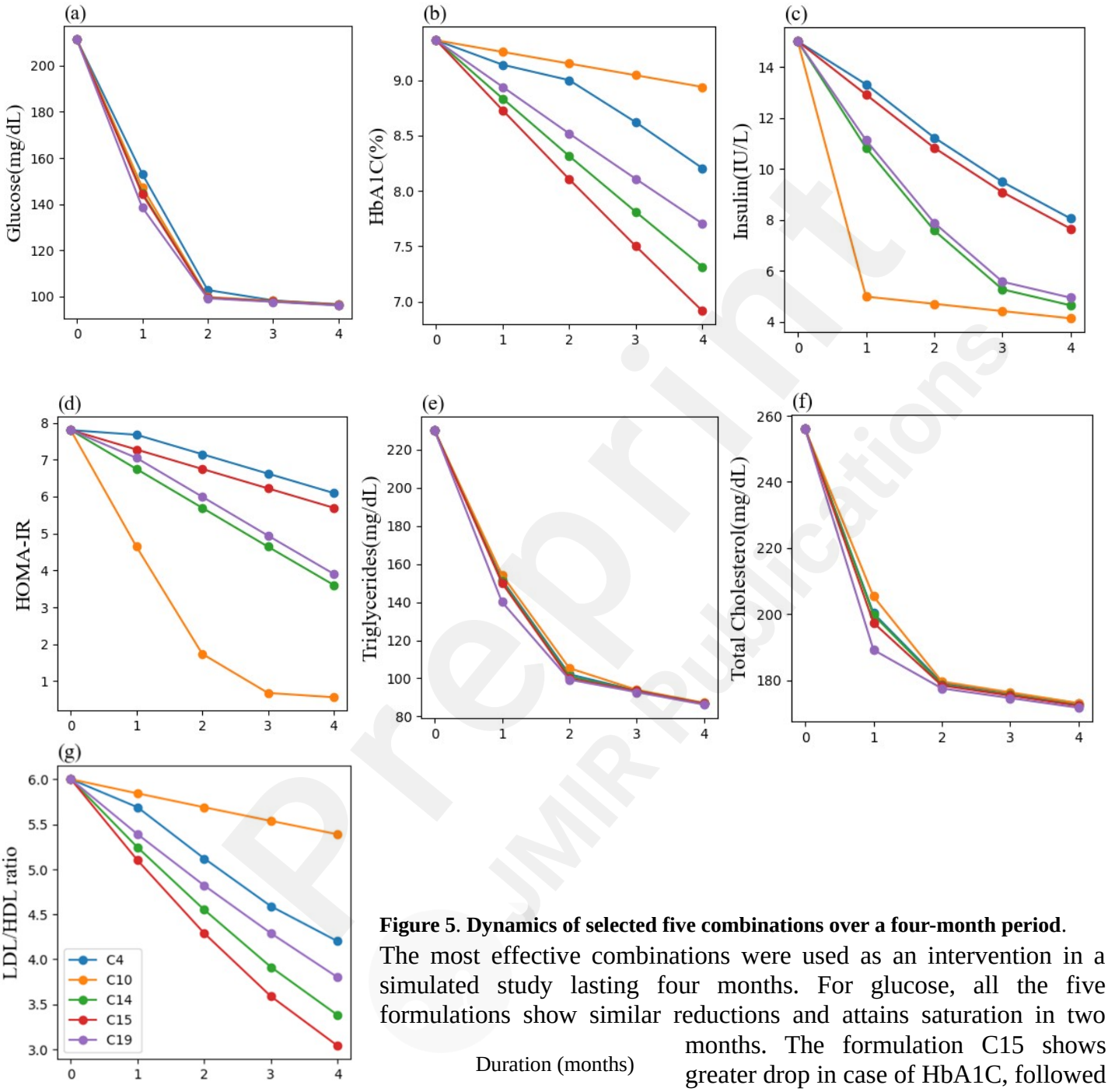


Figure 5. Dynamics of selected five combinations over a four-month period.

The most effective combinations were used as an intervention in a simulated study lasting four months. For glucose, all the five formulations show similar reductions and attains saturation in two months. The formulation C15 shows greater drop in case of HbA1C, followed by C14 and C19. In the case of insulin, the formulation C10 achieves saturation quickly, within approximately one month with the rapid drop compared to the other formulations. The formulation C15 performs well, reaching a steady state within about 3 months in HOMA-IR as well. Formulations C14 and C19 were the next best performing combinations for insulin and HOMA-IR. In case of triglycerides, the formulation C19 shows slightly quicker drop and all the formulation reach a stable concentration in 3 months period. Its cholesterol-modulating properties become evident within about 2 months. The C15 formulation demonstrates prompt drop in LDL/HDL ratio.

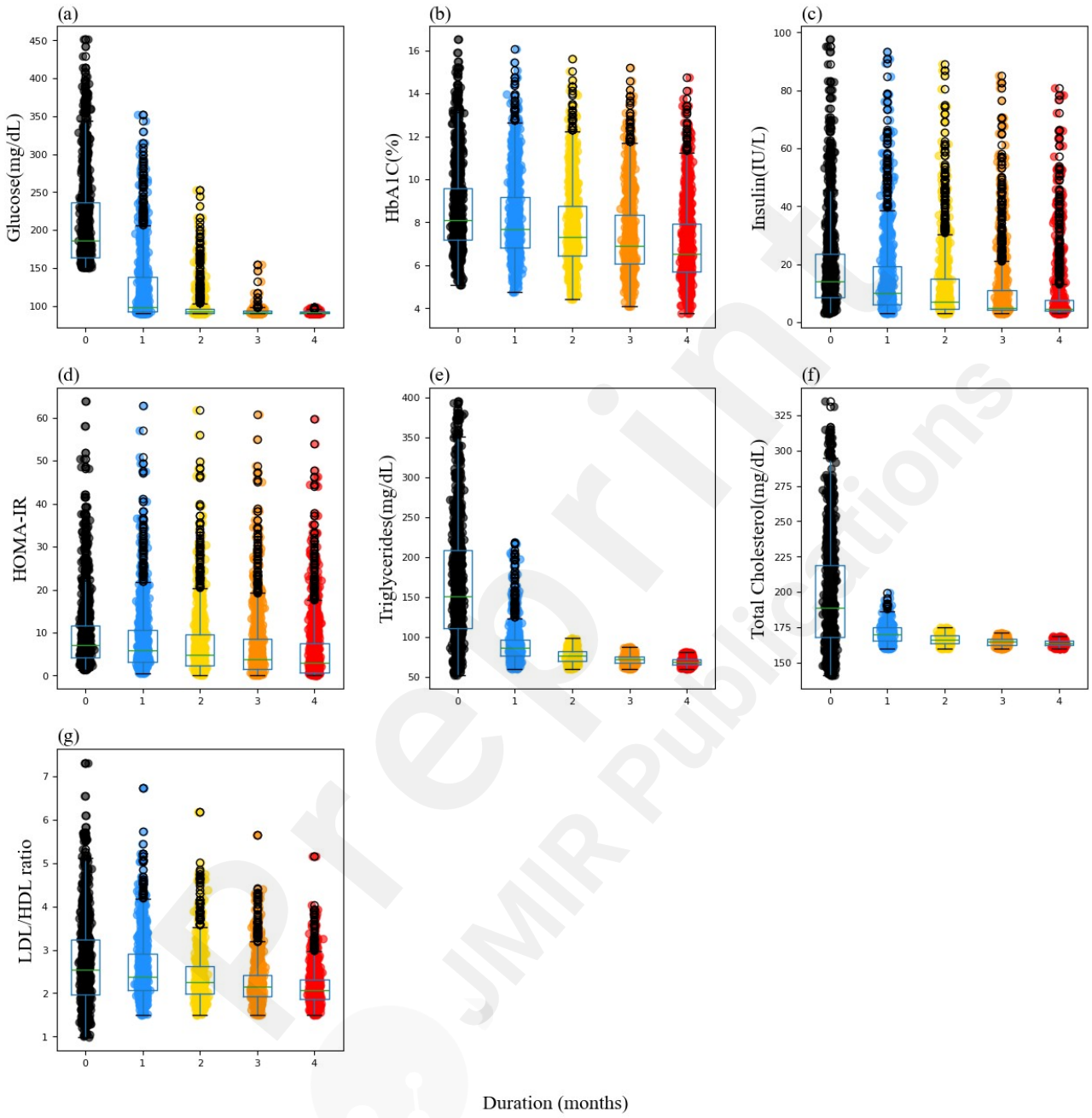
Observations indicate that the C15 formulation leads to significant reductions in glucose,

triglycerides, HbA1C, total cholesterol, and the HDL/LDL ratio. However, this formulation has less effect on insulin and HOMA-IR. Formulation C10 performed well for insulin and HOMA-IR but had least effect on other biomarkers. Whereas the C19 formulation demonstrate optimal performance across all biomarkers and were considered for in silico population analysis. Since the combination C14 also showed a comprehensive effect and was at the same time cost effective, its populations studies have also been performed and studied in sect ion 2 of supplementary material.

Effect of optimal formulation C19 on an in-silico population:

Figure 6. Population Study for the formulation C19: Comparative Analysis of Blood Biomarkers Over 4 months.

The intervention involving formulation C19 led to significant improvements in diabetes-specific



biomarkers when taken daily over varying time periods. These results highlight the positive impact of this formulation on overall health. Specifically, glucose levels decreased from an average of 209 mg/dL to 124 mg/dL within one month, reaching 91 mg/dL by the fourth month. Approximately 92% of the population achieved healthy glucose levels within three months, with values approaching baseline levels over time. Similarly, HbA1C levels declined from 8.6% to 8.2% within one month, eventually reaching 7% by the fourth month. About 20% of the population reached healthy HbA1C levels within four months. Insulin levels also showed improvement, dropping from 19 IU/mL to 15.7 IU/mL in one month and reaching 9.3 IU/mL by the fourth month. Around 63% of the population attained healthy insulin levels within three months. Furthermore, HOMA-IR values decreased from

9.6 to 6.8 over three months, eventually reaching 5.9 by the fourth month. Total cholesterol levels reduced from 196 mg/dL to 170 mg/dL within one month, reaching 163 mg/dL by the fourth month. Remarkably, 99% of the population achieved healthy cholesterol levels within three months. Triglyceride levels followed a similar trend, dropping from 166 mg/dL to 91 mg/dL in one month and reaching 69 mg/dL by the fourth month. Nearly the entire population (99%) achieved healthy triglyceride levels within three months. Lastly, the LDL/HDL ratio decreased from 2.69 to 2.57 within one month, eventually achieving a ratio of 2 by the fourth month. Approximately 64% of the population reached healthy LDL/HDL ratios within four months. Overall, the formulation consistently demonstrated efficacy in normalizing biomarker levels within three months, with further improvements observed over longer durations. A case study was conducted using the C19 combination as an intervention to validate the algorithm.

Case Study on Formulation C19

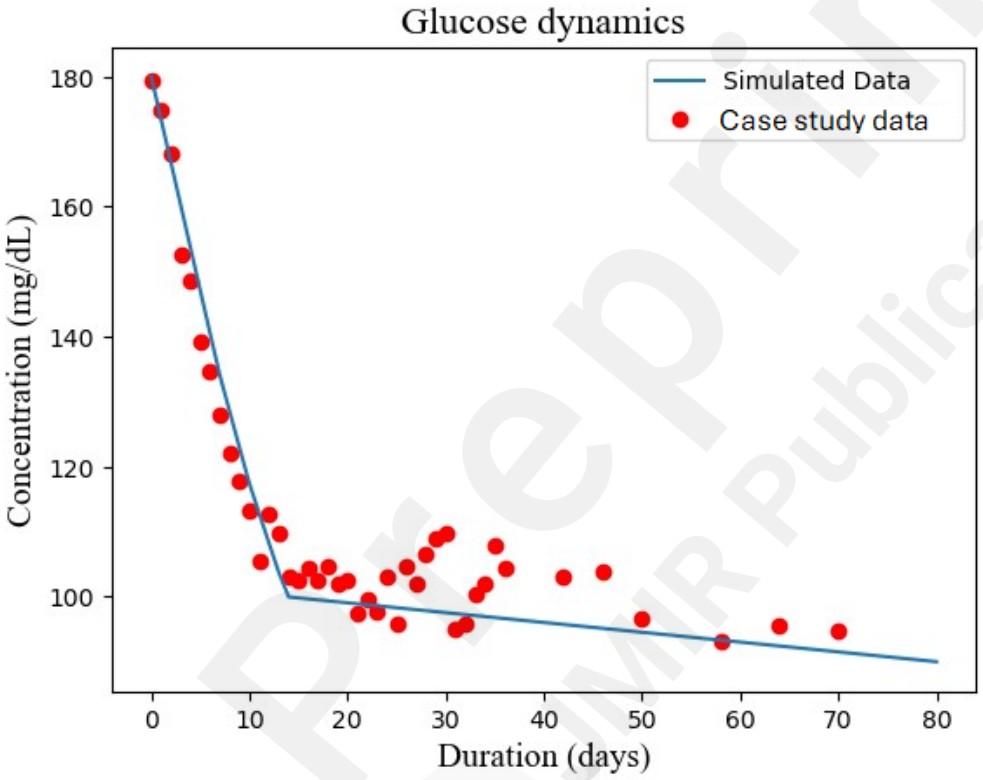


Figure 7. Effect of C19 formulation on daily fasting glucose levels from case study and algorithm prediction. Blue line represents the simulation result, and the red dots indicate the experimental result.

A case study was conducted with a group of ten individuals diagnosed with early-stage diabetes. These participants were following a normal diet and maintaining regular physical activity but were not willing to take any medication. To assess the efficacy of a specific mushroom blend, these individuals were administered combination coded C19 with this being repeated twice daily for 10 weeks. Subsequently the fasting blood glucose levels of the participants were measured and duly recorded. Figure 7 shows a comparison between the actual empirical data obtained from the case study and the predicted glucose levels by model simulation. The simulated data, depicted by a continuous blue line, shows a rapid decline in glucose concentration from approximately 180 mg/dL at the start to around 100 mg/dL by day 20, followed by a gradual decrease over the remaining days. The case study data, depicted by red dots, exhibits a comparable pattern. Initially, the case study data closely aligns with the simulated data, indicating a swift decrease in glucose levels due to the influence of the blend. However, over time, this decline diminishes as the glucose concentration approaches its basal level. This observation suggests that the algorithm also accurately captures the saturation levels of biomarkers. The close correlation between

the simulated results and the actual case outcomes underscores the potential accuracy and reliability of the simulation.

Discussions

Therapeutic Potential of P. ostreatus and P. citrinopileatus in Metabolic Regulation

P. ostreatus has demonstrated a remarkable ability to significantly reduce blood glucose levels and cholesterol levels (Fig. 2), making it effective for managing hyperglycemia and dyslipidemia (Sayeed et al., 2014). Its glucose-lowering effect is attributed to its high content of polysaccharides and phenolic compounds, which enhance insulin sensitivity and glucose uptake, while reducing oxidative stress and improving metabolic functions. Similarly, *P. citrinopileatus* is noted for its ability to lower circulating fatty acids, making it valuable for managing dyslipidemia (Fig.1) (Shu et al., 2006b). Its lipid-lowering properties are due to bioactive compounds like beta-glucans that inhibit lipid synthesis and promote lipid metabolism (Cugnet-Anceau et al., 2010), resulting in improved lipid profiles crucial for cardiovascular health. The combined therapeutic potential of these mushrooms underscores their significance in metabolic regulation and cardiovascular disease prevention.

Synergistic Effects of Mushroom Combinations in Metabolic Regulation

Combining *P. ostreatus* and *P. citrinopileatus* can synergistically improve blood glucose levels and lipid profiles. The enhanced insulin sensitivity from *P. ostreatus*, because of its components like flavonoids(chrysin) and Omega-3 fatty acid (alpha linolenic acid) coupled with the lipid-lowering properties of *P. citrinopileatus*, offers a potent solution for managing both dyslipidemia and blood glucose levels. This combination leverages the strengths of each mushroom species, providing a comprehensive approach to metabolic health. Additionally, a blend of *P. ostreatus* and *P. sajor caju* has shown to be particularly effective in decreasing glucose and insulin levels. *P. sajor caju*, with its components like trehalose and phenols such as myricetin, complements the glucose-lowering effects of P. *ostreatus*, leading to better overall glucose regulation (Finimundy et al., 2018). Furthermore, its impact on reducing amyloid deposition also contributes to enhanced insulin secretion. This combination is especially beneficial for individuals with type 2 diabetes. Incorporating *P. citrinopileatus* into this mix can further enhance glucose regulation while mitigating the effects of dyslipidemia, providing a holistic approach to managing metabolic health and leveraging the unique properties of each mushroom species for optimal health outcomes.

C14 and C15 show Effective Glucose, Lipids and CVD risk Management

The formulation C14 demonstrated a significant reduction in glucose, triglycerides and total cholesterol levels. This can be attributed to the combined effects of *P. ostreatus* and *P. sajor caju*. *P. ostreatus* enhances glucose uptake in muscle and adipose tissues by upregulating GLUT4, thereby effectively lowering blood glucose levels. These mechanisms are possible due to the variety of active components present in these mushrooms, including beta-carotene (carotenoid), glutamine (amino acid), quercetin and catechin (phenols), trehalose (sugar), and coumaric acid. This mechanism is crucial for managing hyperglycemia, a hallmark of Type 2 Diabetes. Additionally, the antioxidant properties of *P. ostreatus* and *P.sajor caju* help reduce oxidative stress, which is often elevated in diabetic patients and can exacerbate glucose dysregulation. The reduction in oxidative stress not only

aids in better glucose management but also protects pancreatic beta cells from damage, ensuring sustained insulin production. This formulation also excelled in lowering total cholesterol levels. This effect can be attributed to *P.ostreatus*. The formulations C15 significantly reduced LDL cholesterol due to higher dose of *P. ostreatus*, which contains lovastatin, an HMG-CoA-reductase inhibitor (Devi et al., 2024; Ehab A.M. EL-Shoura et al., 2015). This reduction lowers cardiovascular disease risk. These formulations also increase HDL cholesterol due to *P. citrinopileatus* and *P. ostreatus*, enhancing lipid metabolism (Fig 1). This balance reduces atherosclerosis risk and other cardiovascular complications in diabetes. C14 and C15 also effectively reduced HbA1c levels, indicating better glucose control. Additionally, *P. ostreatus* and *P. sajor caju's* anti-inflammatory properties protect pancreatic beta cells, improving insulin signalling.

Enhancing Insulin Sensitivity with C10, C5 and C11 formulations

The formulations C10, C5 and C11 markedly reduced insulin levels, demonstrating enhanced insulin sensitivity primarily attributed to the combined effects of *P. sajor caju and P. ostreatus*. These mushrooms enhance beta-cell function and increase GLP-1 secretion, attributed to their contents such as phenolic acids (including alpha-linolenic acid) and flavonoids (such as myricetin), thereby improving insulin regulation and postprandial glucose control. *P. sajor caju* reduces mitochondrial dysfunction and oxidative stress, enhancing cellular energy metabolism and maintaining efficient insulin signalling. *P. ostreatus's* anti-inflammatory properties lower chronic inflammation, restoring normal insulin function and reducing the burden on pancreatic beta cells. The reduced insulin levels suggest enhanced glucose uptake and utilization, crucial for managing Type 2 Diabetes (Fig.1). The formulations C5 and C11, with the lowest HOMA-IR values, showed the greatest improvement in insulin resistance.

Conclusion

In this study, we developed and applied a predictive algorithm to assess the impact of interventions using combinations of three mushrooms (*P.citrinopileautus, P.sajor caju, and P.ostreatus*) on Type 2 Diabetes (T2D) biomarkers. Initially validated against literature data, the algorithm accurately simulated the biochemical effects of these mushrooms on key T2D biomarkers. We used this validated algorithm to predict levels of glucose, HbA1c, insulin, HOMA-IR, LDL/HDL ratio, and total cholesterol following interventions with different mushroom combinations. These predictions were cross validated with case study data, confirming the algorithm’s reliability and precision. Additionally, the algorithm determined the optimal dosages of mushroom combinations for significant reductions in targeted biomarkers while considering formulation costs. Integrating literature data, case study validation, and cost analysis, our approach provides a comprehensive framework for designing effective and economical T2D interventions. This study highlights the potential of computational models in advancing diabetes treatment by optimizing natural therapeutic strategies.

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