World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 3
Page: 512-528

MODULATION BY GOLDENSEAL, BERBERINE, AND METFORMIN ON GLYCOLYTIC FLUX AND MITOCHONDRIAL RESPIRATION: A SEAHORSE XF24 ANALYSIS

***Kiran Kumar Kandunuri and Kenneth White**

School of Human Sciences, London Metropolitan University, 166-220 Holloway Road, London-N7 8DB, United Kingdom.

Article Received: 14 April 2025 | | ***Article Revised: 05 May 2025*** | | ***Article Accepted: 26 May 2025***

***Corresponding Author: Kiran Kumar Kandunuri**
School of Human Sciences, London Metropolitan University, 166-220 Holloway Road, London-N7 8DB, United Kingdom.
DOI: https://doi.org/10.5281/zenodo.15671636

How to cite this Article: Kiran Kumar Kandunuri and Kenneth White (2025) MODULATION BY GOLDENSEAL, BERBERINE, AND METFORMIN ON GLYCOLYTIC FLUX AND MITOCHONDRIAL RESPIRATION: A SEAHORSE XF24 ANALYSIS. World Journal of Pharmaceutical Science and Research, 4(3), 512-528. https://doi.org/10.5281/zenodo.15671636

ABSTRACT

Mitochondrial dysfunction is closely associated with type 2 diabetes, contributing to impaired insulin sensitivity and production. Abnormal mitochondrial activity has been observed in diabetic patients, affecting cellular energy generation. In this study, Seahorse XF analysis was employed to investigate mitochondrial respiration dynamics, including oxygen consumption rate and extracellular acidification rate, reflecting glycolysis. The findings showed that three treatments—metformin, berberine, and goldenseal extract—significantly improved glucose metabolism, increased metabolic flux linked to adenosine triphosphate generation, and raised lactate production rates (p<0.01). Goldenseal extract was less effective in enhancing glucose metabolism compared to the pure alkaloid compound berberine. Despite this, both treatments showed promise for the development of novel oral anti-diabetic agents targeting mitochondrial function and metabolic regulation. These findings align with existing literature, further emphasising the therapeutic potential of these compounds.

KEYWORDS: Mitochondrial dysfunction; Mitochondrial respiration; Oxygen consumption rate; Extra cellular acidification rate; Glycolysis; Adenosine triphosphate generation; Lactate production; Metabolic regulation; Type 2 Diabetes.

1. INTRODUCTION

Mitochondria, the cell's powerhouse, are essential for energy production and play a pivotal role in aging and obesity and serve as focal sites for a wide range of cellular processes, including cellular energy metabolism, ion homeostasis management, redox signaling, and cell death. Common consequences of mitochondrial malfunction include a preference for glycolysis over oxidative phosphorylation (OXPHOS), increased reactive oxygen species production, and aberrant mitochondria-mediated apoptotic machinery.[1,2,3,4] They are closely associated with insulin resistance in type 2 diabetes (T2D).[5]

Mitochondrial dysfunction has been linked to the development of T2D, as it contributes to both reduced insulin sensitivity and impaired insulin production.[5] This diminished cellular responsiveness to insulin, combined with defective secretion and action, further exacerbates mitochondrial dysfunction. However, the exact molecular mechanisms underlying these processes remain unclear.[6] Tissues with high mitochondrial content, such as skeletal muscle, liver, brain, and heart, are particularly affected.[7,8] Studies reveal T2D-associated changes in mitochondrial gene expression and enzymatic activity, including decreased expression of genes like PGC1A and NRF1 involved in mitochondrial maintenance.[9,10] Additionally, alterations in mitochondrial enzyme activity and content have been reported.[11]

In skeletal muscle of adults with obesity and/or T2D, decreased mitochondrial enzymatic activity, respiratory capacity, and fatty acid oxidation are observed, mirroring findings from T2D animal models.[12,13] Reduced OXPHOS, particularly at complex I and III, has been documented in diabetic mouse models.[13] While skeletal muscle is the primary tissue studied, insulin resistance impacts other tissues, including liver, adipose tissue, brain, cardiac muscle, and gastrointestinal system. These tissues exhibit distinct phenotypes, although the underlying molecular impairments often share common pathways.[7,14,15]

Research has shown that diabetic patients' pancreatic β-cells have abnormal mitochondria, affecting their energy generation. A study published in Science by University of Michigan researchers found that dysfunctional mitochondria impact the maturation and function of β-cells in mice.[16] The researchers impaired three key mitochondrial functions: DNA repair, removal of damaged mitochondria, and maintenance of healthy mitochondria. This triggered a stress response causing β-cells to become immature and stop producing sufficient insulin. The findings showed that mitochondria can signal the nucleus and alter cell fate, which was confirmed in human pancreatic islet cells.[16] The research revealed that reversing mitochondrial damage could potentially aid in curing diabetes irrespective of cell type. The researchers observed that mitochondrial damage did not result in cell death[16], suggesting the possibility that reversing this damage could restore normal cellular function[16], and aberrant mitochondrial structure, mtDNA genetic mutations, alterations in energy metabolism (Warburg effect), and isocitrate dehydrogenase enzyme mutations.

Targeting faulty signaling cascades in the mitochondria, a variety of natural substances have demonstrated efficacy in correcting faulty metabolism. While some natural substances modify metabolic anomalies resulting from mitochondrial dysfunction, others operate indirectly by directly targeting the components of the mitochondria.[17] The current study provides mechanistic insight into mitochondrial metabolism and treatment processes of the promising natural substances goldenseal and berberine as compared to the most sought-after biguanide class of medicine metformin, which targets mitochondrial malfunction.[18]

Goldenseal (*Hydrastis canadensis* L.) is a medicinal plant widely recognised in traditional medicine and as a dietary supplement. The rhizomes and roots of this plant have long been utilised to address various health conditions. Both *in vitro* and *in vivo* studies of goldenseal extract have revealed promising therapeutic properties, including enhanced glucose uptake, GLUT-1 stimulation, as well as anti-diabetic and weight-loss effects.[18,19] Berberine, a natural compound derived from the rhizomes of *Hydrastis canadensis L.* or *Rhizoma Coptidis*, has demonstrated promising pharmacological effects. Preliminary studies suggest that its ability to regulate mitochondrial activity underlies its diverse benefits, including improved glucose uptake, blood sugar regulation, lipid metabolism, and weight reduction. However, the exact mechanisms through which berberine influences mitochondrial function remain to be elucidated. Current research proposes that berberine primarily affects glycolipid metabolism by modulating mitochondrial respiratory chain activity. These findings[18,19,20] offer valuable insights into the mitochondrial basis of berberine's effects, providing a robust scientific groundwork for its potential use in clinical treatments. Given these findings, goldenseal and berberine have been selected for further investigation into their influence on mitochondrial respiration when compared to the biguanide class of anti-diabetic agent metformin.

Mitochondrial dysfunction affects many cell types, including liver cells, leading us to study HepG2 cells exposed to goldenseal extract, berberine, and metformin to see if they improve cell metabolism. In this investigation, the Seahorse Bioscience XF24 high-throughput metabolic analyzer (Massachusetts, USA) was used to monitor the treated HepG2 cells' mitochondrial function in real time. Based on the rate of change in the pH and dissolved oxygen in the cell culture media, as well as concurrently nearby viable adherent HepG2 cells cultured in a 24 well microtitre plate in real time mode, the XF cell Mito stress Kit is an optimised solution that was used in this study to analyse mitochondrial activity. This method determines the rate of treated HepG2 cellular metabolism in detail, including the oxygen and proton exchange rates, for short periods of time (5 minutes) and with a tiny volume of medium.[21] The analyser's 24 optical fluorescent heads were fixed in disposable sterile cartridges on a 24-well microtitre plate. As soon as the optical heads enter the wells and become engrossed in the medium, they send optical signals independently and simultaneously with other optical heads. The experiment runs every 2 to 5 minutes and measures oxygen consumption rate (OCR) in pmol/min, which indicates mitochondrial respiration, and extracellular acidification rate (ECAR) in mpH/min, which represents glycolysis.[21]

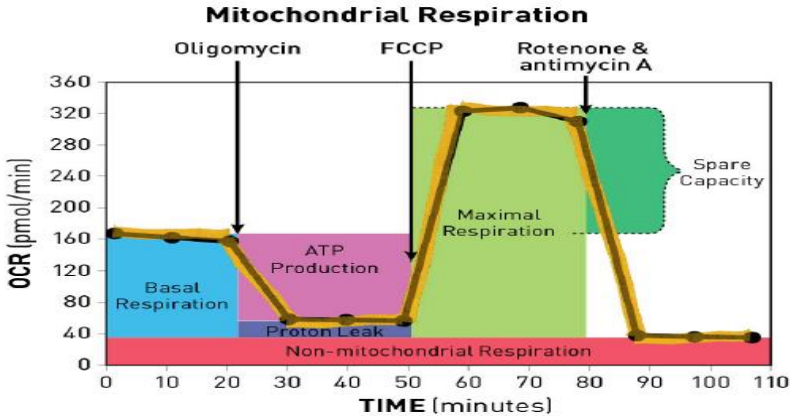

Fig. 1: XF Cell Mito Stress Test. A sketch of the essential parameters of mitochondrial respiration. Sequential compound injections are used to quantify basal cell respiration, adenosine triphosphate (ATP) generation and proton leak, maximal respiration and spare respiratory capacity, and non-mitochondrial respiration.[22]

The XF Cell mito stress experiment included three respiration modulators, each of which acts separately and specifically on a certain target complex of the electron transport chain (ETC). Since oligomycin inhibits the ATP synthase (complex V) enzyme, the OCR in mitochondrial respiration would decrease significantly.[21,22] This effect is directly linked to the production of ATP. As the uncoupling modulator, carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) disengages the mitochondrial membrane's potential and eliminates the proton gradient across the membrane. As a result, respiration was no longer connected to the production of ATP, and the electron transport chain's electron discharge was regulated by the oxygen's availability, resulting in complex IV oxygen consumption reaching its maximum.[21,22]

The third modulator was made up of two components, rotenone, which acts as a complex I inhibitor, and antimycin A, which acts as a complex III inhibitor. This combination exclusively inhibits mitochondrial respiration and allows measurement of non-mitochondrial respiration. Also, the spare respiratory capacity of the cells, is the cells' response to increased energy demand and supply, and it can be measured from the difference between maximal respiration and basal respiration of the cells, using the FCCP-stimulated OCR (Fig 2).[21,22]

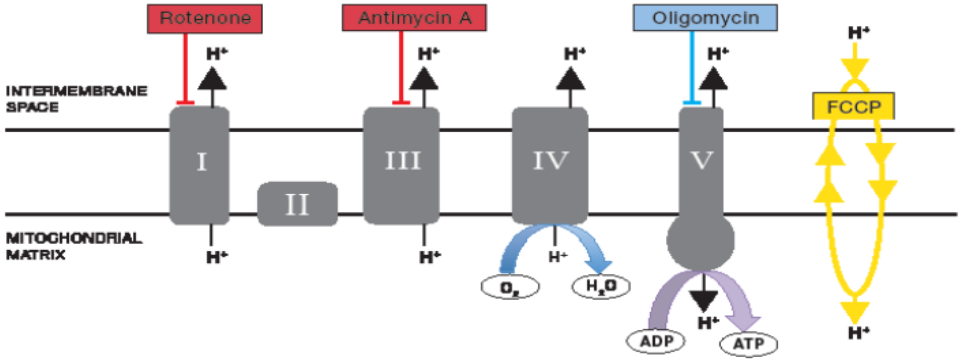

Fig. 2: Electron Transport Chain Modulators in XF Cell Mito Stress Experiment. The electron transport chain complexes and the purpose of the substances in the XF Cell Mito Stress Test Kit are shown in this image. Rotenone/antimycin A inhibits complex I and III, oligomycin inhibits ATP synthase (complex V), and FCCP uncouples oxygen intake by ATP synthesis.[23]

2. MATERIALS AND METHODS

Compounds and Extracts: Berberine chloride hydrate and metformin hydrochloride were sourced from Sigma Aldrich, prepared as 100x stock solutions in DMSO and used at 1000 µM (metformin), and 10 µM (berberine), with 1% (v/v) DMSO as control. Goldenseal tincture was from Napiers the Herbalists, Edinburgh, Scotland. Concentrations of 1% (v/v) were prepared using DMEM media, with respective solvent controls of 0.6% (v/v) ethanol, refelecting the solvent content of the tincture.

The following reagents and consumables were sourced as specified: XF24-3 FluxPak (cat. no. 102070-001) and XF Cell Mito Stress Test Kit (cat. no. 101706-100) from Seahorse Bioscience; uridine, penicillin/streptomycin, DMEM, glucose, glutamine, sodium pyruvate, buffering agent, and HEPES buffer from Sigma-Aldrich; trypsin from Invitrogen; and fetal bovine serum from HyClone.

Cell Culture and Treatments: In a controlled incubator set at 37°C, 95% humidity, and 10% CO_2, confluent HepG2 cells were kept in 75-cm2 T-flasks with DMEM, 10% FBS, and 22 mM glucose. The cells were further subcultured after being detached from the flasks every three days using a 0.25% trypsin solution. At the time of subculturing, the cells had less than 75% confluence. The cells were seeded at 30,000 cells/well in an XF24 cell culture microtitre plate. The cells were incubated for two nights with controls (1% (v/v) DMSO, and 0.6% (v/v) ethanol), and goldenseal ethanolic extract (1% (v/v)), berberine (10 µM), and metformin (1000 µM). Following the phyto treatments, basal respiration of the cells, proton leak, and ATP turnover after applying oligomycin (1 µM) to inhibit ATP formation, maximal mitochondrial respiratory capacity by adminstring FCCP (carbonyl cyanide 4-trifluoromethoxy phenylhydrazone) (1 µM) as an uncoupler, and non-mitochondrial respiration by applying rotenone and antimycine (1 µM) to break mitochondrial respiration were measured (OCR).[22]

Assay Procedure: After removing the treated cells' cell culture media from the 24 wellplates, 500 µl of Seahorse media was added to each well, and the cells were washed. After that, 525 µl of Seahorse media was added to each well, and the cells were incubated in the laminar flow hood for 45 minutes. The concentrations of pyruvate and glucose in the Seahorse medium were 1 mM and 10 mM, respectively. Using the crystal violet staining assay (Crystal violet staining cytotoxicity assay Kit; BioVision, Inc., Milpitas, California, USA), the number of cells was determined at the conclusion of the assay. Using triplicate or quadruplicate samples, the OCR readings were divided by the number of cells per well.

Crystal Violet Staining Assay: This assay is a quick and accurate way to measure living cells. Viable cells can be measured by staining with crystal violet dye, which causes the dead cells to separate from the wells' bottoms. Because it binds to both proteins and DNA, the quantity of crystal violet staining in a culture is closely correlated with the number of live cells. Reagents and consumables included Crystal violet (0.2%) (triarylmethane dye), paraformaldehyde (4%), sodium dodecyl sulfate (5%) and phosphate buffered saline (PBS) (Crystal violet cytotoxicity assay Kit; BioVision, Inc., Milpitas, California, USA).

Following the aspiration of the medium from the 24 well plates, 50 µL of PFA (4% paraformaldehyde) was applied to each well. The PFA was aspirated after ten minutes of rocking, and PBS was used to wash the cells. After adding 25 µL of 0.2% crystal violet to each well, the cells were rocked for ten minutes before being cleaned with PBS once again. Following the addition of 100 µL of SDS (5%) to each well, the cells were rocked for five minutes. 50 µL of samples were aliquoted into a 96-well plate and absorbance measured at 570nm using a Fluostar Omega microplate reader (BMG Labtech, UK).

Statistical Analysis: The experimental data is presented as the mean + standard deviation (S.D.) of *n* independent measurements. Statistical significance was calculated as the difference between the control and the target treatments using the student's t-test and one-way ANOVA with Bonferroni's multiple comparison post hoc tests. Microsoft Excel was used for these analyses. Siginificance thresholds were defined as $p>0.05$ regarded as (not significant), $*p<0.05$, $**p<0.01$, $***p<0.001$, and $****p<0.0001$. Also, the Agilent Seahorse XF Cell Mito Stress Test software and Wave software was used to analyse and present the data.

3. RESULTS

In vitro and *in vivo* studies showed that goldenseal and berberine significantly influence glucose metabolism in HepG2 cells, indicating an anti-diabetic effect by enhancing glucose uptake through an increase in glucose transporter-1.[18,19] This raises the question of whether berberine or goldenseal might also alter the rate of glucose metabolism, potentially leading to a mild uncoupling of OXPHOS. This could allow for the breakdown of glucose without producing energy. An analysis was performed to assess the effects of berberine and goldenseal on glucose metabolism in comparison to metformin, using the Seahorse system. The Seahorse instrument employs fluorescence probes to continuously track oxygen consumption and the production of extracellular acidification, which is believed to result from lactate formation. By applying specific inhibitors that target various stages of the electron transport chain, one can determine the role of mitochondria compared to other cellular processes in oxygen consumption.[22]

The diagram from Fig 1 and the matching profile of oxygen consumption of HepG2 cells exposed to the treatments are reproduced in Fig 4 to aid in data interpretation. The experiment analysed mitochondrial respiration in HepG2 cells treated with goldenseal extract, berberine, and metformin using the Seahorse XF24 analyzer. HepG2 cells were cultured under controlled conditions and exposed to treatments for 48 hours, including goldenseal ethanolic extract (1% (v/v)), berberine (10 μM), and metformin (1000 μM), along with solvent controls such as 1% (v/v) DMSO and 0.6% (v/v) ethanol. After the treatment, the Seahorse XF24 analyzer measured OCR and ECAR to evaluate mitochondrial respiration and glycolysis, using Seahorse media containing glucose (10 mM) and pyruvate (1 mM). Specific modulators like oligomycin, FCCP, and a combination of rotenone and antimycin A were employed to quantify basal respiration, ATP-linked respiration, maximal respiration, proton leak, and non-mitochondrial respiration. To normalise OCR readings, a crystal violet cytotoxicity assay was performed to determine viable cell count per well (Fig 3), ensuring accurate comparisons across treatments. Data from three independent experiments were analysed using the Agilent Seahorse XF Cell Mito Stress Test software[40] and statistical methods such as ANOVA and Bonferroni post hoc tests to assess mitochondrial coupling efficiency, glycolysis, and ATP generation. The study aimed to understand how these treatments modulate mitochondrial function and potentially contribute to therapeutic strategies for T2D. Fig 5 displays the graphs from the output.

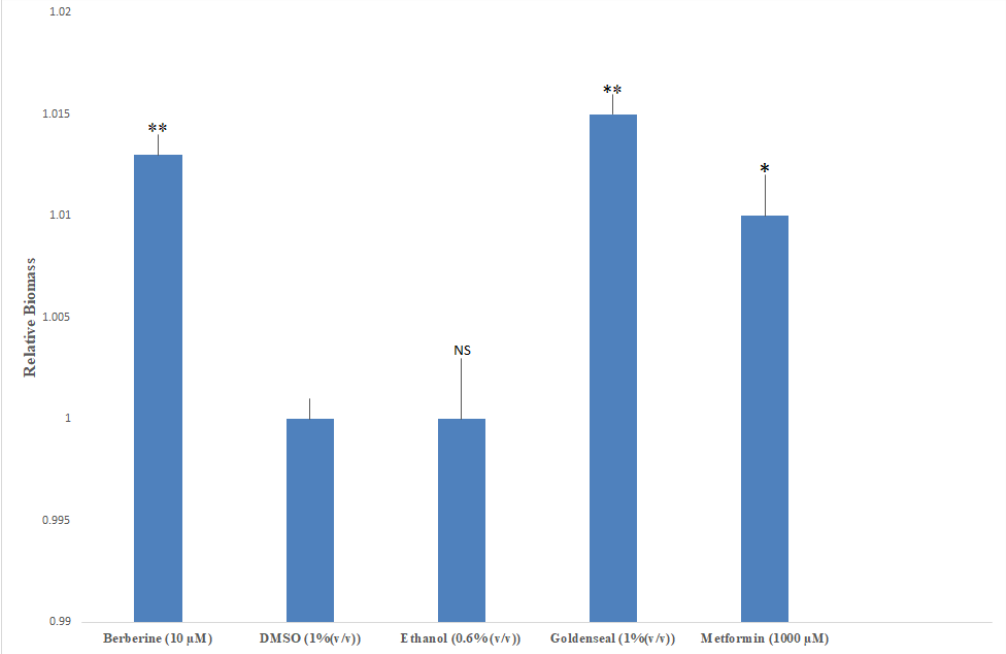

Fig. 3: The Relative Biomass of HepG2 Cells after Long-Term Treatment. Goldenseal ethanolic extract, berberine, and metformin were used to treat HepG2 cells for 48 hrs, with ethanol and DMSO serving as solvent controls. The data are relative cell mass, normalised to the DMSO control, and represent the mean + SD of three independent trials. In each experiment, the treatments were repeated three times. One-way ANOVA with Bonferroni's multiple comparison post hoc test was used to determine significant differences between control and target treatments ($p>0.05$(NS), $*p<0.05$ and $**p<0.01$, respectively). The therapies had modest impact on the cell number.

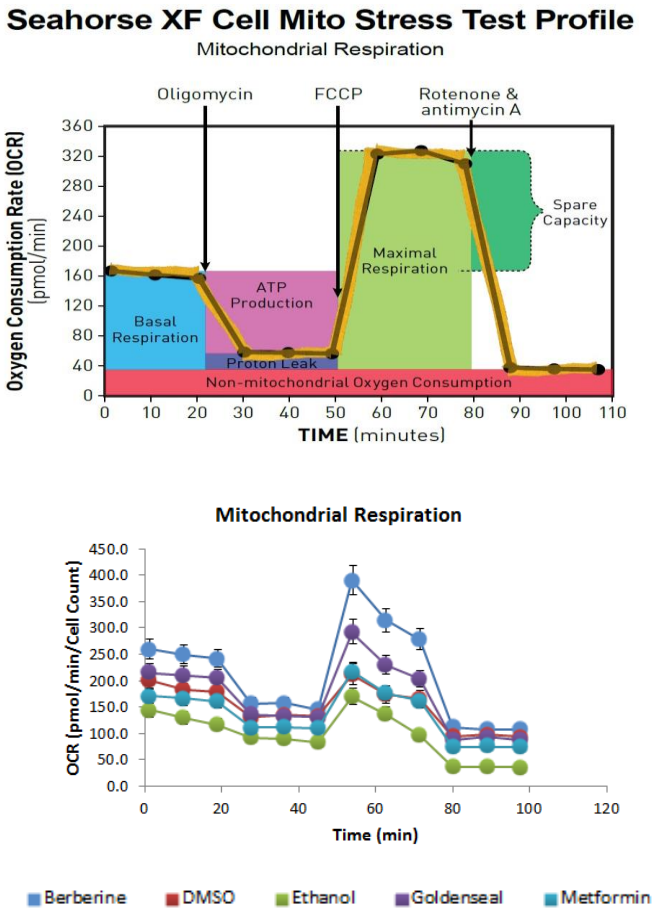

Fig. 4: Treatment Effects on Mitochondrial Respiration in HepG2 Cells. The bottom chart illustrates the OCR of mitochondrial respiration measured in HepG2 cells subjected to long-term treatment. Data represent the mean ± SD of three independent experiments (n = 3). Statistical analysis was performed using Student's t-test, with significant differences observed between groups ($p < 0.05$).

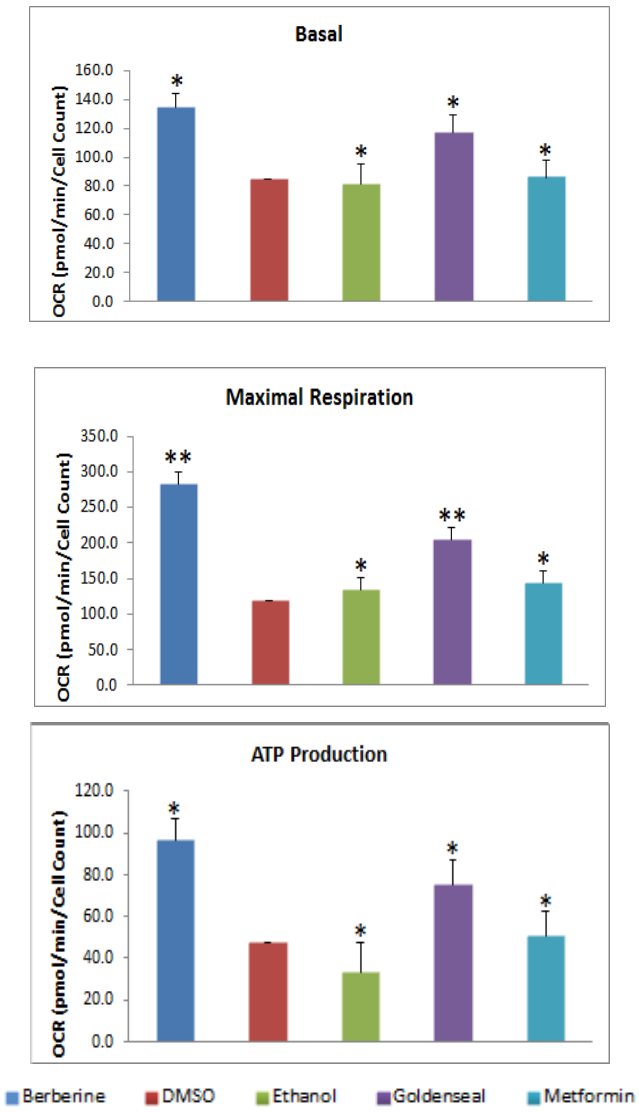

Fig. 5: Basic Metabolic Parameters. The OCR of long-term treated HepG2 cells were measured for basal respiration (top), maximal respiration (middle), and ATP generation (bottom). The data shown is the mean + SD of three measurements (student's t-test *p<0.05).

The data in Fig 5 reveal that berberine and goldenseal both promote the rate of mitochondrial metabolism, as seen by higher ATP generation and maximal respiration when compared to solvent controls. Metformin has a similar impact, albeit less pronounced.

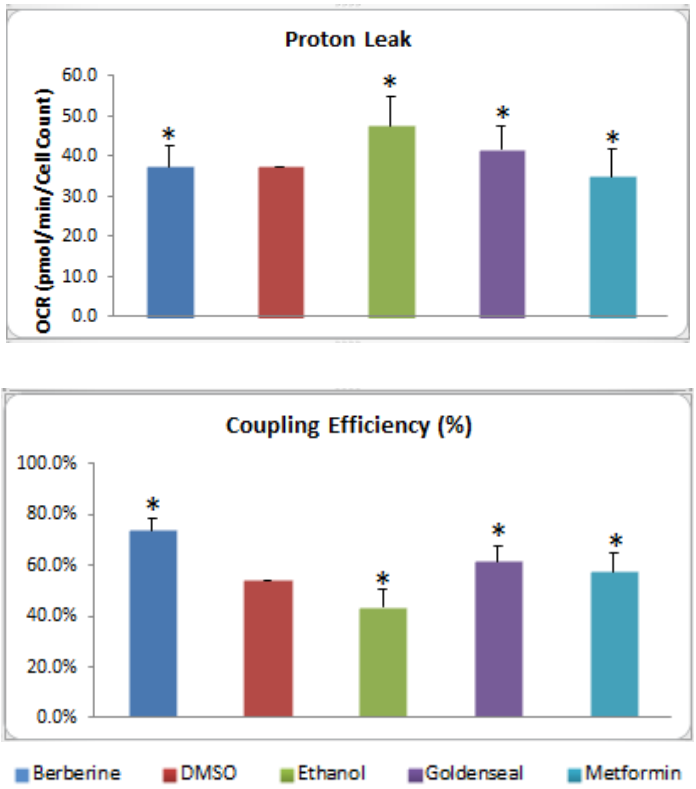

Fig. 6: Measurement of Uncoupling. OCR assessment of the proton leak (the top chart), and coupling efficiency (the bottom chart), of long-term treated HepG2 cells, the data are the mean +SD from three measurements (student's t-test *$p<0.05$).

The findings in Figure 6 show how treatments affect the connection between the electron transport chain and ATP generation. If there is increased leakage of protons back across the inner membrane of the mitochondria, this indicates uncoupling, which is reflected in a reduced coupling efficiency. The studies show that berberine and goldenseal reduce proton leakage and increase coupling efficiency. Finally, the Seahorse study provides information on glycolysis rate, as illustrated in Fig 7. There is variation among the treatments, all of which increase glycolysis in comparison to control cells. Goldenseal and berberine increase glycolysis rates by 100-125% compared to controls, while metformin reduces glycolysis by roughly 50% compared to controls. These results support a higher glucose flux through cells treated with goldenseal, berberine, or metformin.

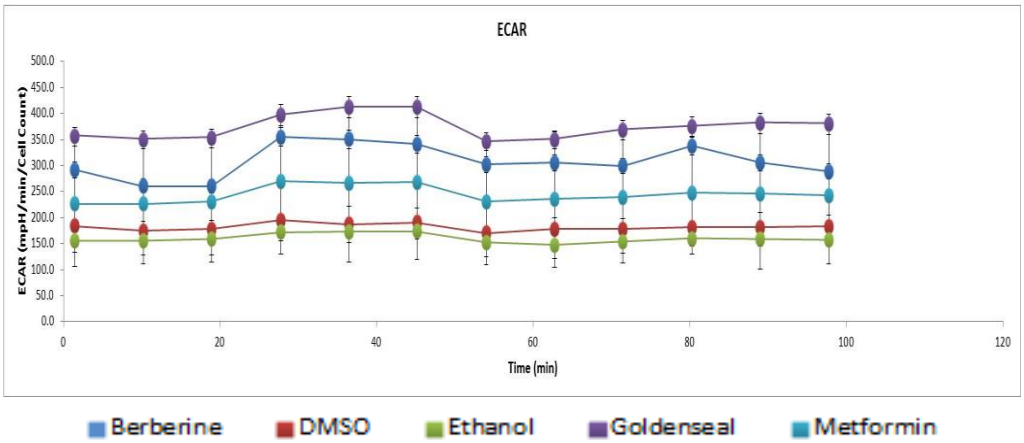

Fig. 7: The Glycolytic Rate of HepG2 Cells following Treatment. The ECAR was assessed in cells subjected to long-term treatment. Data represent the mean ± SD from three independent measurements. Statistical significance of differences between the groups was determined using Student's t-test, with $p<0.01$ indicating significant differences.

4. DISCUSSION

Mitochondria, often considered the energy generators of the cell, are essential for sustaining cellular function and facilitating growth and proliferation. These organelles convert nutrients into ATP, the primary energy currency of the cell, through the process of OXPHOS. Their pivotal role extends beyond energy production, influencing processes such as signaling, cellular differentiation, and the regulation of the cell cycle, and synthesizing macromolecular precursors necessary for biological functions. However, mitochondria face a unique challenge—managing competing metabolic pathways within the same space. On one hand, OXPHOS primarily relies on intermediates from the tricarboxylic acid (TCA) cycle to generate ATP. On the other hand, the synthesis of amino acids like proline and ornithine uses a reductive process. Until now, scientists were puzzled about how mitochondria navigate these competing processes without compromising either.[24] Recent research sheds light on this mystery. Studies reveal that pyrroline-5-carboxylate synthase (P5CS), the enzyme essential for producing proline and ornithine, is selectively concentrated in certain mitochondria that lack cristae and ATP synthase. This segregation ensures that reductive biosynthesis can occur without interfering with OXPHOS. The process is driven by P5CS's ability to form filaments and by the mitochondrial fusion and fission cycle—dynamic processes that shape mitochondrial structure and function. When the fusion and fission cycle is disrupted, the separation between mitochondria specialized for OXPHOS and those for proline synthesis breaks down. Cells are then forced into a trade-off: either sustaining reductive synthesis at the expense of efficient ATP production, or prioritizing OXPHOS while sacrificing proline synthesis.[24]

This discovery highlights the critical role of mitochondrial dynamics in maintaining cellular balance. By segregating pathways through fusion and fission, mitochondria adapt to fluctuating nutrient availability and energy demands, supporting both oxidative and reductive biosynthetic processes. These findings open new avenues for understanding how cellular metabolism responds to environmental changes and may have implications for studying diseases linked to mitochondrial dysfunction.[24]

Mitochondria perform a remarkable division of labour to balance competing metabolic demands. These organelles segregate into specialized subpopulations: one dedicated to energy generation through OXPHOS and the other to biosynthetic activities, such as proline production. This functional specialization is driven by the enzyme P5CS, which

forms filaments under specific nutrient conditions to enhance biosynthesis. Mitochondria containing P5CS filaments lack ATP synthase and the cristae structures vital for efficient OXPHOS, highlighting their distinct roles. Through dynamic fusion and fission processes, these mitochondrial subpopulations are spatially and functionally organized, enabling cells to adapt to shifting energy needs while sustaining reductive biosynthesis. This discovery sheds light on the intricate mechanisms by which cells allocate resources among metabolically distinct mitochondrial populations.[25]

When mitochondrial respiration is increased, cells may require additional energy substrates, such as glucose, to meet energy demands, potentially leading to increased glycolysis. Citrate, which can be exported to the cytosol and converted into acetyl-CoA, which feeds into the glycolytic pathway, can be produced at higher levels through improved mitochondrial respiration. Additionally, enhance NADPH production, which is required for the Pentose Phosphate Pathway (PPP). The PPP generates NADPH and ribose-5-phosphate, which are important for antioxidant defense and nucleotide synthesis, respectively. This may result in increased glycolysis in order to supply the necessary precursors.[41] Improved mitochondrial respiration can activate signalling pathways such the P13K/Akt pathway, promoting glycolytic gene expression and increasing glycolysis. The link between mitochondrial respiration and glycolysis varies according to the cell type, tissue, and physiological context. In some malignant cells, enhanced mitochondrial respiration can coexist with increased glycolysis.[26]

To further investigate the effects of goldenseal and berberine on glucose metabolism in HepG2 cells, measurements of oxygen consumption and lactate generation were performed using the Seahorse XF-24 analyzer[21], which can monitor the metabolism of living cells. The data imply that increased stimulation of glucose uptake by berberine and goldenseal is associated with increased metabolism, which can be linked to an increase in glucose storage, necessitating an increase in metabolic flux of ATP synthesis.

Berberine and goldenseal both accelerate mitochondrial metabolism by increasing ATP generation and enhancing maximum respiration as compared to controls. Metformin has similar qualities but is less effective (Fig 5). In a study, metformin was shown to enhance mitochondrial function, increasing respiration, membrane potential, and ATP production in hepatocytes. Clinically relevant doses of metformin improved mitochondrial density and complex I activity in the liver, accompanied by reduced hyperglycemia in high-fat diet (HFD)-fed mice. Through activation of 5' AMP-activated protein kinase (AMPK), metformin promoted mitochondrial fission, thereby optimizing mitochondrial respiration and rejuvenating the mitochondrial lifecycle. However, HFD-fed mice with liver-specific knockout of AMPKα1/2 subunits exhibited higher blood glucose levels under metformin treatment, highlighting the central role of AMPK in its mechanism of action. While metformin activation of AMPK improved mitochondrial respiration and hyperglycemia in obesity, supra-pharmacological concentrations of metformin disrupted adenine nucleotide balance, leading to a cessation of mitochondrial respiration.[27] Notably, in this study, metformin significantly enhanced mitochondrial respiration compared to controls ($*p<0.05$).

It was interesting to observe how the treatments affected the coupling between ATP synthesis and the electron transport chain, as well as if increased proton leakage across the inner mitochondrial membrane resulted in uncoupling. This could be one mechanism by which treatments might improve the metabolism of glucose. The observations, however, indicate that berberine and goldenseal do not work by uncoupling OXPHOS but rather increase metabolic activity, as they produced an increased coupling efficiency (Fig 6).

Goldenseal and berberine enhance glycolysis rates to 100–125% above control levels, while metformin-treated cells exhibit glycolysis approximately 50% higher than controls (Fig 7). This observation, coupled with increased electron transport coupling, is noteworthy. Significant differences may exist in these parameters when comparing primary cell cultures and cancer cells, as mitochondrial behavior in cancer cells often diverges from that in primary cultures.[18,28,29,30]

The three treatments (metformin, goldenseal extract, and berberine) yielded similar results. Berberine has been shown to promote glucose absorption in HepG2 cells up to 50 µM.[31] However, a recent study found that 15-60 µM berberine inhibited HepG2 cell growth and decreased glucose consumption.[32] The cause of the disparity between the two investigations is unclear. The current study's findings indicate that berberine is well tolerated by HepG2 cells and may even boost their proliferation. Berberine enhances glucose intake by increasing glycogen storage while decreasing glucose release. Metformin had similar effects in the current investigation, which is consistent with previous findings.[18] for HepG2 cells. In another study, while dimethylbiguanide reduces oxygen consumption and mitochondrial membrane potential in intact rat hepatocytes, it has no effect on isolated mitochondria or permeabilized rat hepatocytes.[33] Metformin has also been shown to inhibit respiratory chain complex I activity in isolated liver mitochondria prepared from 24-hour-starved Wistar rats.[34]

Berberine was observed to dose-dependently inhibit respiration in L6 myotubes and muscle mitochondria derived from Wistar rats. This effect was attributed to a specific inhibition of respiratory complex I, akin to the mechanisms noted with metformin and rosiglitazone.[35] The preliminary findings of this study diverge from prior *in vivo* observations, potentially due to several factors: the use of intact hepatoma cells rather than isolated mitochondria, variations in concentration and experimental protocols, the complex composition of the goldenseal root extract, or differences in the time course utilised in this investigation. Moreover, Seahorse XF assays were performed without comprehensive optimisation owing to time constraints, which may have influenced the outcomes.

Seahorse XF analysis revealed findings consistent with the literature, indicating an enhanced rate of lactate production across the three treatments.[31] This elevation contributes to increased glucose metabolism. Lactic acid, a byproduct of glucose metabolism under anaerobic conditions, is frequently linked to muscle fatigue and conditions such as lactic acidosis. Nevertheless, lactic acid fulfills a crucial function in metabolic processes. During intense physical activity or stress, when oxygen availability is limited (anaerobic conditions), lactic acid is produced as a byproduct of glucose metabolism. Under anaerobic glycolysis, pyruvate, derived from glucose breakdown, is converted to lactic acid to regenerate NAD^+, thereby sustaining ATP production. Additionally, lactate—originating from lactic acid—serves as an energy source by being transported to organs such as the liver, where it undergoes conversion back into glucose through the Cori cycle. This cycle underscores lactate’s pivotal role in preserving energy balance during oxygen-limited conditions.[36,37]

A recent study published in Nature Metabolism explored the role of lactate in human carbohydrate metabolism. The study examined 15 healthy, physically active adults (8 women, 7 men) and demonstrated that after carbohydrate ingestion, lactate rapidly enters the bloodstream before glucose. This finding highlight lactate's role as a major energy carrier and buffer, working with insulin to regulate blood glucose levels. Contrary to its association with anaerobic metabolism, lactate appears to serve as a normal metabolic response to carbohydrate ingestion, both at rest and during exercise. Using isotope-labeled tracers, the study revealed that dietary glucose is rapidly converted into lactate in the

intestines, contributing to the body's energy distribution. Furthermore, lactate serves as a preferred fuel in skeletal muscle, heart, and brain, outperforming glucose under certain conditions. These findings provide evidence that elevated lactate levels reflect physiological energy flux rather than a toxic byproduct.[38]

Goldenseal and berberine both compounds enhance mitochondrial respiration, as evidenced by increased OCR and ATP generation (Fig 4 & Fig 5). This suggests improved mitochondrial function. Interestingly, they also increase ECAR, glycolysis, and lactate production (Fig 7). This dual effect could be due to their ability to stimulate glucose uptake and metabolism, leading to higher glycolytic flux alongside enhanced OXPHOS. The increased lactate production might reflect a compensatory mechanism to maintain energy balance under conditions of heightened metabolic activity.

Goldenseal and berberine appear to improve coupling efficiency in the electron transport chain, reducing proton leakage (Fig 6). This indicates that their effects are not due to uncoupling OXPHOS but rather optimizing mitochondrial function. Enhanced glycolysis could be linked to their impact on glucose transporter expression or glycolytic enzyme activity.

Berberine improves glycolysis in HepG2 cells but requires the presence of AMPKα1. Berberine has been shown in studies to promote glucose consumption and lactate release in HepG2 cells, both of which indicate improved glycolysis. However, knocking down the AMPKα1 gene in these cells fully eliminates berberine's capacity to activate glycolysis.[39] Berberine raises the phosphorylation of AMPK α1 and total AMPKα in wild type HepG2 cells, which raises AMPK activity. For berberine to have an impact on glucose metabolism, particularly glycolysis, AMPK activation is essential. The study confirmed the crucial function of AMPKα1 in berberine's action by finding that AMPKα1-knockout cells were unable to experience berberine's stimulating effects on glucose consumption and lactate release.[39]

Metformin also improves mitochondrial respiration but to a lesser extent compared to goldenseal and berberine (Fig 5). It activates AMPK, promoting mitochondrial fission and optimizing respiration. However, metformin reduces glycolysis compared to controls (Fig 7), which contrasts with the effects of goldenseal and berberine. This reduction might be due to its inhibitory action on respiratory complex I. The interplay between mitochondrial respiration and glycolysis in these treatments highlights their potential as anti-diabetic agents.

HepG2 cells are a good model for investigating liver cell metabolism, although they are not a perfect substitute for real liver cells due to their malignant origin. Because of their different metabolic and signaling pathways, these cells could not behave exactly like native hepatocytes. This highlights the importance of carefully interpreting results, particularly when thinking about translation to clinical or *in vivo* settings.[28,29,30]

Goldenseal's notable enhancement of glycolysis may be due to its impact on the expression of glucose transporters.[4] or the activity of glycolytic enzymes. Furthermore, the metabolic change seen in treated cells may be attributed to goldenseal's impact on mitochondrial enzymes or energy-regulating pathways like AMPK. To elucidate these pathways and validate their applicability to *in vivo* settings, more research is required.

5. CONCLUSION

The observed simultaneous improvement in mitochondrial respiration and glycolysis can be explained by the treatments' ability to enhance glucose uptake and overall metabolic activity in HepG2 cells. Compounds like goldenseal extract and berberine stimulate glucose transporter activity[18], increasing glucose influx into the cells. This elevated glucose flux supports both OXPHOS (mitochondrial respiration) for ATP generation and glycolytic activity, functioning as a complementary pathway to accommodate increased energy demands. Notably, the treatments optimise mitochondrial function by improving coupling efficiency in the electron transport chain, thereby reducing proton leakage rather than uncoupling OXPHOS. The simultaneous rise in glycolysis and mitochondrial respiration likely reflects a coordinated metabolic adaptation to ensure efficient energy production and balance under increased metabolic flux. This dual enhancement underscores their potential as therapeutic agents for conditions like T2D.

6. ACKNOWLEDGEMENTS: We extend our thanks to Dr Nick Jones and Prof Venkat Kanamarlapudi of Swansea University's Institute of Life Science for providing the facilities and technologies essential to this study.

7. CONFLICTS OF INTEREST: The authors hereby declare that there are no conflicts of interest.

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