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1TestRequirement:

- In vitro Screening of anti-diabetic adjuvant activity of Dietary Supplement
- The samples to be tested as follows:

S. No	Customer Name	Compound Name	Cell Line
1	**HOLIN AWARD NUTRACEUTICALS LLP CHENNAI**	**DIABOVITA (DIABETIC CARE)**	**$HepG_2$**

2 Method:

$HepG_2$ cells were cultured in DMEM containing 4.5 g/L D-glucose with 10% heat-inactivated FBS at 37°C, 5% CO2 atmosphere. The cells were trypsinized and cells were counted by tryphan blue assay and seeded into 96-well plates with 5000cells per well with six wells left as blank wells and let growing for three days. After three days of seeding, Standard Metformin with the combination of diabovita was added in the concentration of 5,10,25,50 and 100 µg/ml in triplicates. After 48 hr incubation, the spent culture medium was aspirated and replaced with a 25 µl incubation buffer (DMEM diluted with PBS, 0.1% BSA and 8 mm glucose) and further incubated for an additional 3 h at 37° C. 0.1 µg/ml.Untreated contained only the incubation buffer without diabovita used as the negative control. After incubation, 10 µl of the incubation medium was removed from each well and transferred into a new 96-well plate into which 200 µl of glucose oxidase reagent was added to determine the concentration of glucose in the medium. After 15 min of incubation at 37° C, the absorbance was measured at 492 nm using a Multi-scan plate reader. The amount of glucose utilized was calculated as the difference between the cell-free and cell-containing wells. The percentage of glucose utilization was calculated in relation to the untreated controls.

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3 Statistical Analyses

All the data were analysed statistically by one-way ANOVA analysis of variance and Dunnett's post-test and were expressed as mean ± SD. The level of statistical significance was set at $p < 0.05$.

4 Results:

Studied the glucose uptake of diabovita concentrations of 5 to 100 µg/ml with metformin 0.1 µg/ml.The diabovita increasing of concentration from 5 to 100 µg/ml increases the glucose utilization in $HepG_2$ cells in dose dependent manner compared to untreated control **(Table 1 & Figure 1).**

Extract (µg/ml)	OD	Glucose Utilization (% Control)	SD
Control	0.628	100	1.25
Metformin	1.032	164.33	1.92
Metformin+5 Diabovita	1.046	166.56	2.41
Metformin+10 Diabovita	1.071	170.54	2.72
Metforrmin+25 Diabovita	1.119	178.18	3.18
Metformin+50 Diabovita	1.134	180.57	3.46
Metformin+100 Diabovita	1.163	185.35	4.31

Table 1: Effect diabovita on glucose utilization in $HepG_2$ cells. Cells were treated in the presence or absence of varying concentration of the diabovita. Data expressed as mean ± SD (n = 3).

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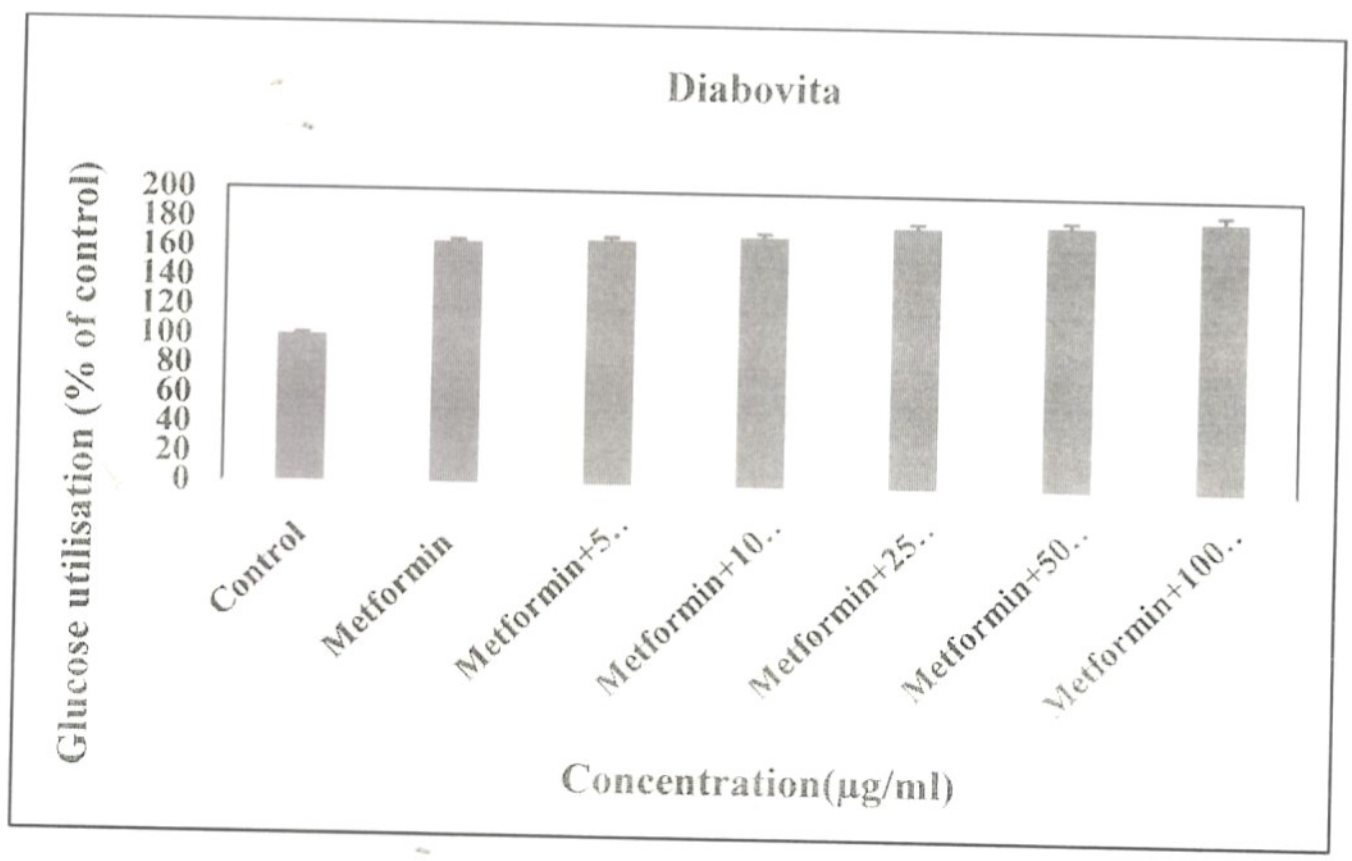

Figure 1: Effect diabovita on glucose utilization in $HepG_2$ cells. Cells were treated in the presence or absence of varying concentration of the diabovita. Data expressed as mean ± SD (n = 3).

5 Reference

1. Samuel Odeyemi and John Dewar. In Vitro Antidiabetic Activity Affecting Glucose Uptake in HepG2 Cells Following Their Exposure to Extracts of Lauridiatetragona (L.f.) R.H. Archer. Processes 2020, 8, 33; doi:10.3390/pr8010033.

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