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The Combined Effect of Berberine and Quercetin against Non-Alcoholic Fatty Liver Disease

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Abstract

The aim of the present study was to explore the protective effect of Berberine and Quercetin against non-alcoholic fatty liver disease. The phrase "non-alcoholic fatty liver disease" refers to a number of liver disorders that can afflict persons who use little to no alcohol. There is currently no effective treatment for NAFLD, and the underlying molecular mechanism that causes this transformation is unclear. Growing interest has been shown in developing natural compound-based therapeutics for metabolic illnesses. Natural supplement berberine is well known for its effective Antibacterial, Antiprotozoal, and Antitrachoma properties in addition it has Hepatoprotective. An essential natural Flavonoid called quercetin has attracted medical interest because of its possible positive effects on human health. It can reduce blood cholesterol levels, inflammation, reduce hepatic fat build up, and regulate abnormalities of the gut microbiota. The MTT test was used to determine if the combination of the substances was more effective against NAFLD than either berberine or quercetin taken separately.HepG2 cells were exposed to a combination of BBR and quercetin at various doses in five different concentration .This study's findings comes to the conclusion that quercetin and berberine together had demonstrated efficacy against cell lines. The combination, individual chemicals, and Rf value of berberine and quercetin all shown to be genuine. The FTIR measurement ultimately demonstrated that there is no herbal- herbal interaction. Therefore, further research is required before moving forward with in vivo investigations using animal models and developing the medicine for clinical assessment before marketing the herbal product for NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a condition in which deposition/accumulation of a fatty substance in the liver occurs without alcohol abuse. It is a global prevalence of 25% and is a leading cause of cirrhosis and hepatocellular carcinoma. The principal risk factors are obesity, non-insulin-dependent diabetes mellitus, and hyperlipidemia. NAFLD has a wide clinical spectrum, ranging from asymptomatic steatosis to steatohepatitis, fibrosis, and cirrhosis. Treatment is the transplantation of the liver and has a bidirectional association with components of the metabolic syndrome. Nonalcoholic fatty liver disease (NAFLD) has grown from a relatively unknown disease to the most common cause of chronic liver disease in the world, with 25% of the world's population thought to have it. Advanced liver fibrosis is a key prognostic marker for liver-related outcomes and overall mortality, and can be assessed with combinations of non-invasive tests. There is currently no approved therapy for NAFLD, although several drugs are in advanced stages of development. Healthy lifestyle and weight reduction remain crucial to the prevention and treatment of NAFLD. NAFLD and NASH are not only found in adults, but also in children and adolescents. The prevalence of these diseases is predicted to increase, causing a tremendous clinical and economic burden and poor patient-reported outcomes. To understand the global trajectory of this disease, an international group of

experts came together during the 2017 American Association for the Study of Liver Diseases Global NAFLD Forum.

2. Materials And Methodology

Both Berberine and quercetin were purchased from Southern scientific suppliers and were stored in the pharmacology laboratory at SRM College of Pharmacy.

Ftir Analysis:

FTIR analysis was used to identify functional groups in a sample. The infrared spectroscopy spectrum (IR) was obtained using FTIR Bruker alpha E & T instrument. The sample was scanned from 4000-500 cm-1 and the percentage of transmittance versus wave number was recorded.

Hptlc Analysis:

Solutions of berberine and quercetin (1000 µg/mL) were separately prepared by dissolving 10 mg accurately weighed standards in small amounts of methanol and making up the volume up to 10 mL in a standard volumetric flask. Then the combination sample was prepared using equal amounts of Berberine and quercetin.

HPTLC analysis was performed on 20×10 cm aluminum-backed plates coated with a 0.2 mm layer of silica gel 60 F254 (Merck,Mumbai,India). Dilutions of standard and test solutions were applied to the plates as bands 5.0 mm wide, 10.0 mm apart, and 10.0 mm from the bottom edge on the same chromatographicplate by using a CAMAG (Muttenz, Switzerland) Linomat sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28±20C) with toluene-ethyl acetate- methanol-formicacid (6:6:2:1% v/v)as mobile phase. The plates were dried in air and scanned at 350 nm with a CAMAG TLC scanner with Win cat software and using a deuterium lamp. The method was validated according to the ICH guidelines.

Method Validation

Validation studies ensure the suitability and reproducibility of the method in analyzing the desired analyte. The method was validated for linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), specificity, and precision (repeatability) as per the International Conference on Harmonization of guidelines. Linearity was determined by averaging the values obtained from calibration curves by regression analysis. LOQ was expressed as 3.3 σ /slope of the calibration curve, whereas LOQ was 10 σ/slope. To estimate LOD and LOQ values,a blank solution (methanol) was spotted six times following the same method as explained above. Application of developed method: test samples (4 µL) were applied in triplicate and chromatograms were obtained.The area under the peak was recorded and the content of the same was calculated from the regression equation obtained from calibration curves.

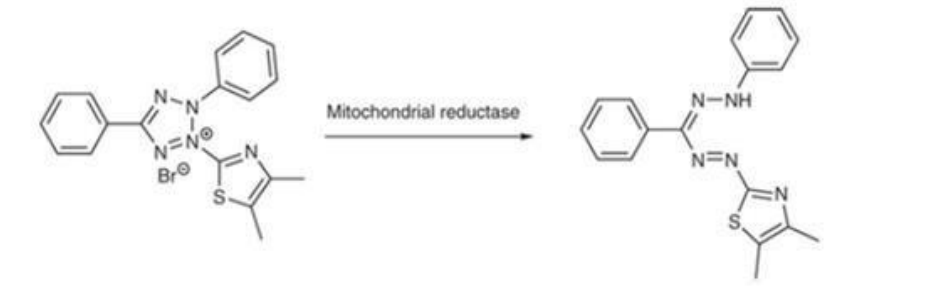
MTT ASSAY

MTT assay is performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The key component is (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide). Dissolved MTT is converted to an insoluble purple formazan, which can be solubilized using DMSO, acidified isopropanol, or other solvents. The result is a measure of cytotoxicity caused by the test material.

Materials And Method

MTT reagent (the solution is filtered through a 0.2µm filter and stored at 28°C for frequent use or frozen for extended periods)

1. DMSO
2. CO2incubator
3. Micro Plate reader
4. Inverted microscope
5. Refrigerated centrifuge



(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))

MTT

(2E,4Z)-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan

(Formazan)

Preparation Of Culture Medium And Test Solution

For the MTT assay, serial two-fold dilutions (6.25 – 100 µg) were prepared from this assay. Cell lines and culture medium were procured from NCCS and the stock cell was cultured in a medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO2 at 37oC until confluent. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/mL using respective media containing 10% FBS. After 24 h, the supernatant was removed and 100 µL of different concentrations of test samples were added to the partial monolayer in microtiter plates. After incubation, the test solutions were discarded and 20µL of MTT was added to each well. The absorbance was measured using an a microplate reader at a wavelength of 570nm. The percentage of viability was calculated using the following formula, % viability = Sample abs/Control abs x100.

3. Result And Discussion

Ftir Analysis

FTIR analysis is performed to ensure that there is no interaction between the taken samples and there are no modifications in their chemical properties and mechanism of action. The results of FTIR peak values and functional groups of samples are represented in the figure (1, 2, 3). IR spectrum of sample-1 shows absorption peaks at 3415.29 cm-1(-OH, Alcohol or phenol), 2926.78 (-CH, Alkane), 2731.01 (-CH, Aldehyde &Ketone), 2724.48 (-C-C, Cycloalkanes), 1663.68 (-C=C, Alkene) and absorption peaks at 3399.00 cm-1 (-OH, Alcoholorphenol), 2921.21 (-CH,Alkane), 2857.71 (-CH, alkane), 2167.34 cm-1 (NH), 2163.27cm-1 (CN), 1635.02 (C=C) for sample 2 and sample 3 shows absorption peaks at 3399.90 (-OH, Alcohol or phenol), 2921.78 (-CH, Alkane), 2731.09 (-CH, Aldehyde &Ketone), 2167.71 (-C-C, Cycloalkanes), 1662.02 (-C=C,Alkene).

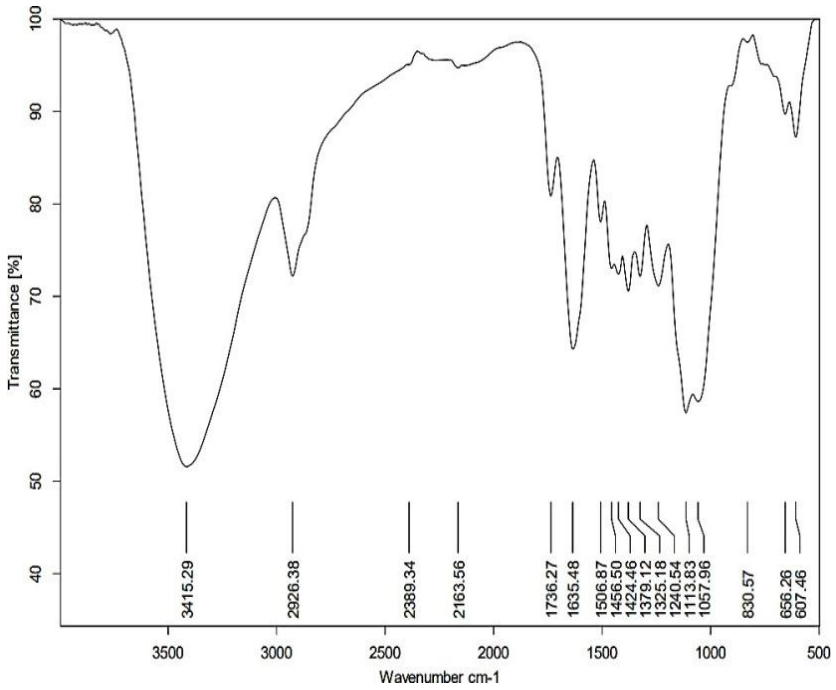


Fig 1: FTIR spectra of Berberine

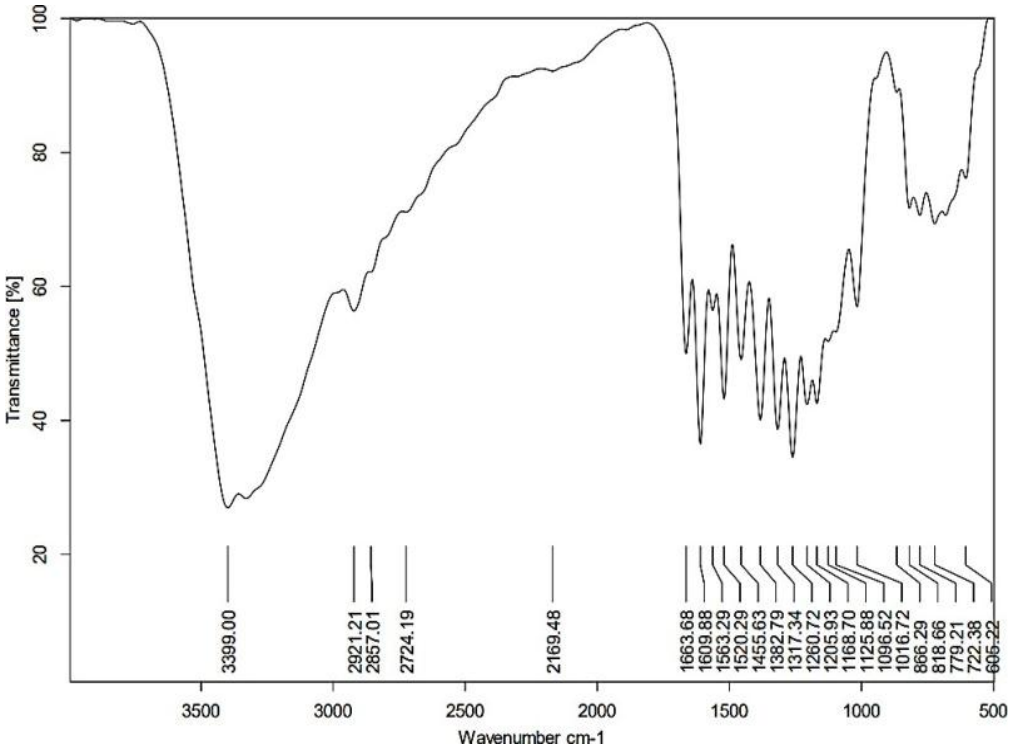


Fig 2: FTIR spectra of qucertine

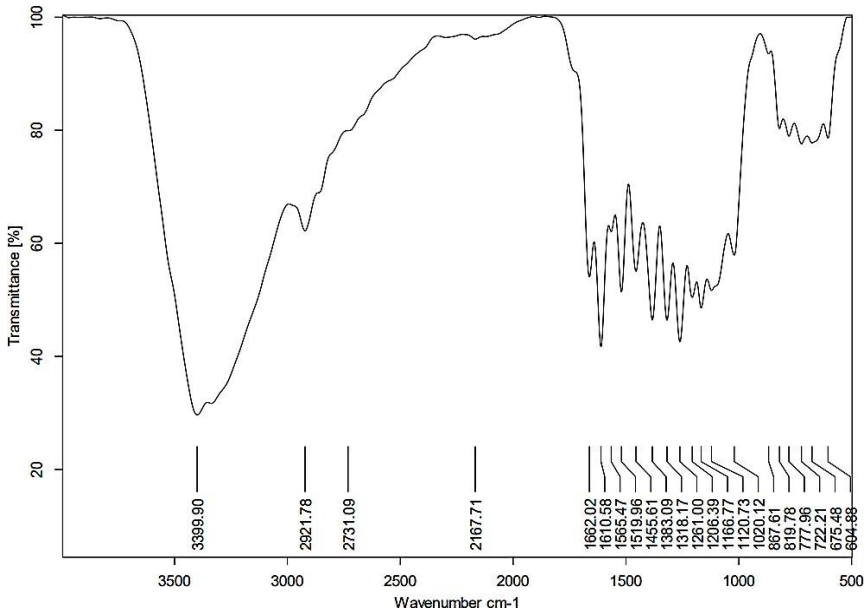


Fig 3: FTIR spectra of the combination of Berberine and quercetin

Hptlc Analysis

HPTLC analysis revealed that the samples are pure compounds, with no significant difference in the Rf values of the individual berberine, quercetin, and the combination of both. The Rf value of Berberine was found to be 0.84, and Quercetin 0.80. The method developed was validated using the calibration curve method, with a concentration range of 0.1–15.0 µg/ml per spot for Berberine and 3.0–50.0 for the combination. The LOD and LOQ values were also linear for all the samples.

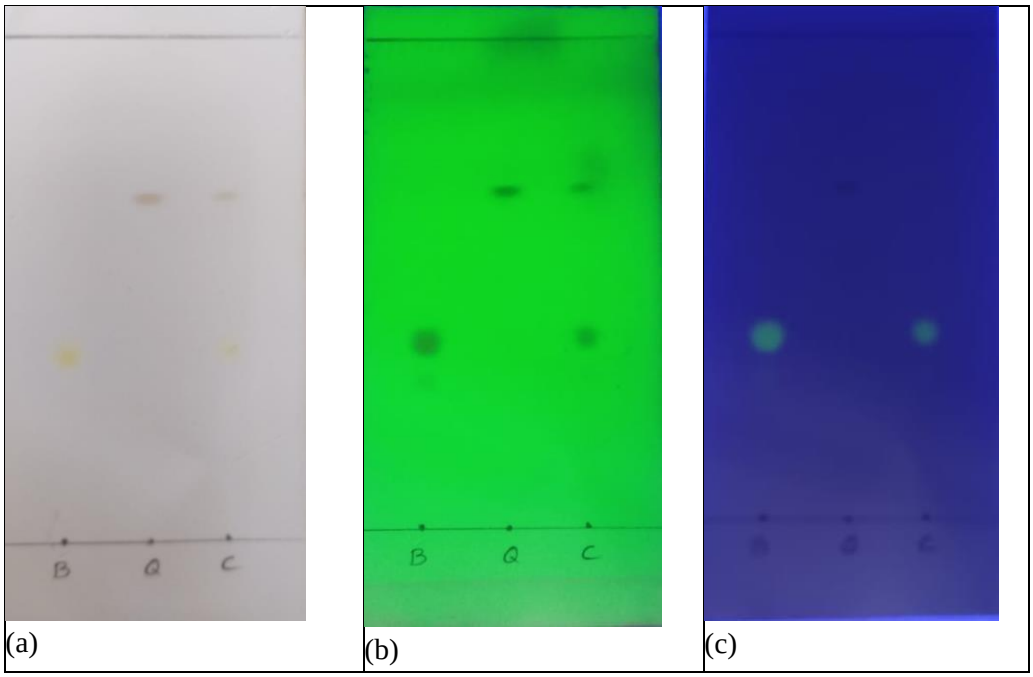


Fig 4: Photograph of chromatograms obtained, at (a) 550 nm – Visible light, (b) 254 nm – UV light(c) 366 nm – Fluorescent light

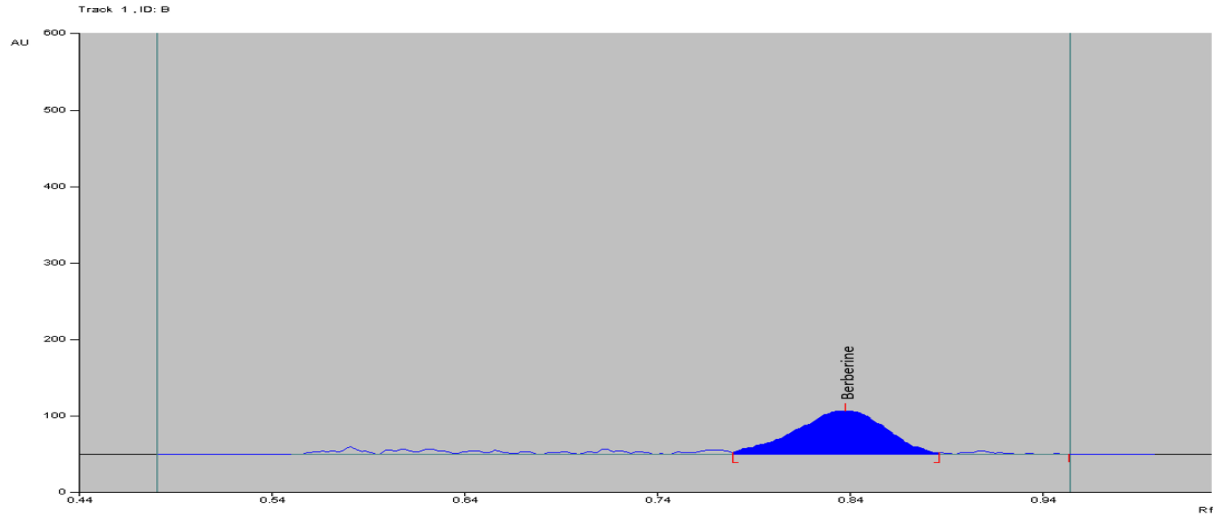


Fig 5: Hptlc Chromatogram Of Berberine

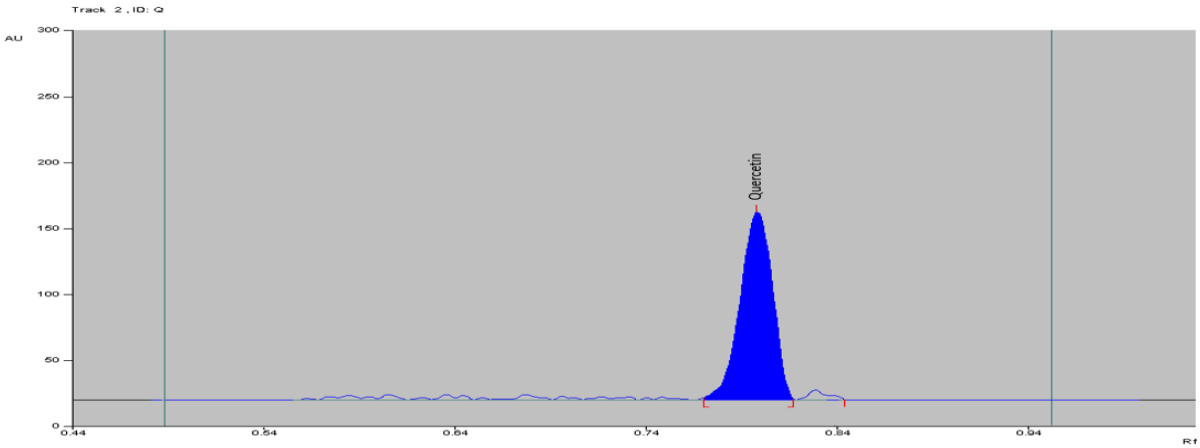


Fig 6: Hptlc Chromatogram Of Qucertine

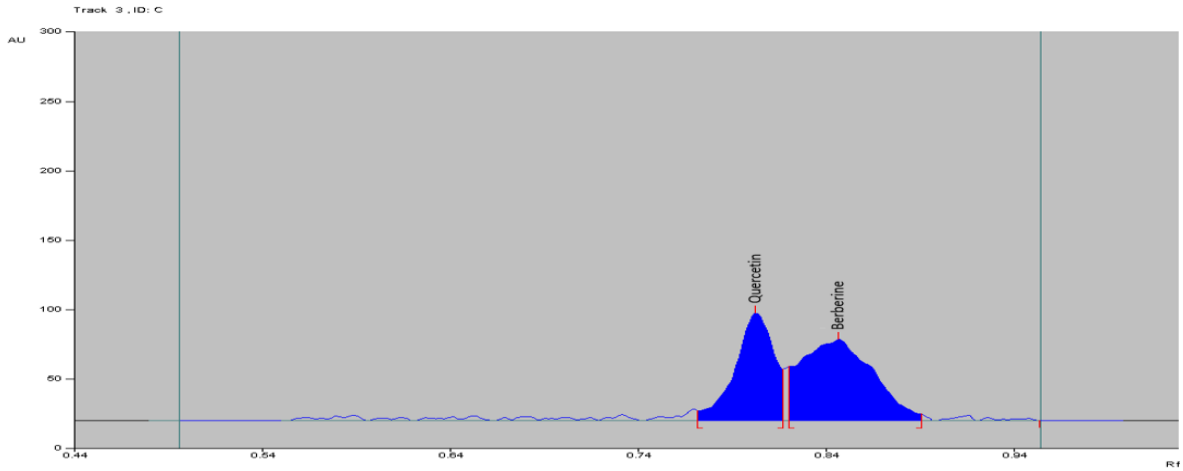


Fig 7: Hptlc Chromatogram Of Berberine And Quercetin

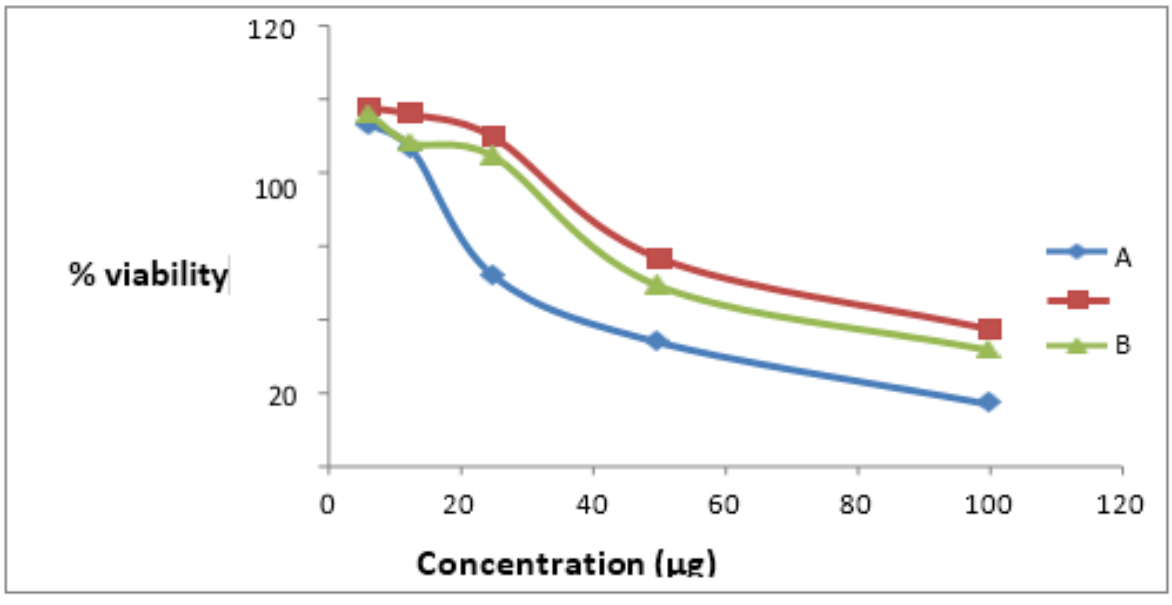
MTT ASSAY:

The MTT assay was performed to evaluate that the combination of the samples has more efficacy against NAFLD than the individual sample of Berberine and quercetin. Sample a = Combination of Berberine and Quercetin, Sample B = Berberine, Sample c =quercetin. The IC50 value of the given test sample (A, B, and C) was found to be 30.92 µg, 75.02 µg, and 56.65 µg, respectively. The combination of the compounds was more effective against NAFLD, with an IC50 of 30.92 µg/ mL as compared to 75.02 µg /ml and 56.65 µg/ml for Berberine and Quercetin, respectively.

Table-1 Study on individual and combined Phytomolecule against HEPG2 cell line in MTT

MTT ASSAY (OD value)			
Concentration µg/ml	**A (Quercetin &Berberine)**	**B (Berberine)**	**C (Quercetin)**
6.25	92.69230769	97.6923077	95.49450549
12.5	86.15384615	95.9340659	87.85714286
25	51.64835165	89.6703297	84.23076923
50	33.73626374	56.5934066	49.06593407
100	17.03296703	37.1978022	31.64835165
IC 50	**30.92 µg/ml**	**75.02 µg/ml**	**56.65 µg//ml**

Fig 8: Represent percentage cell viability versus concentration



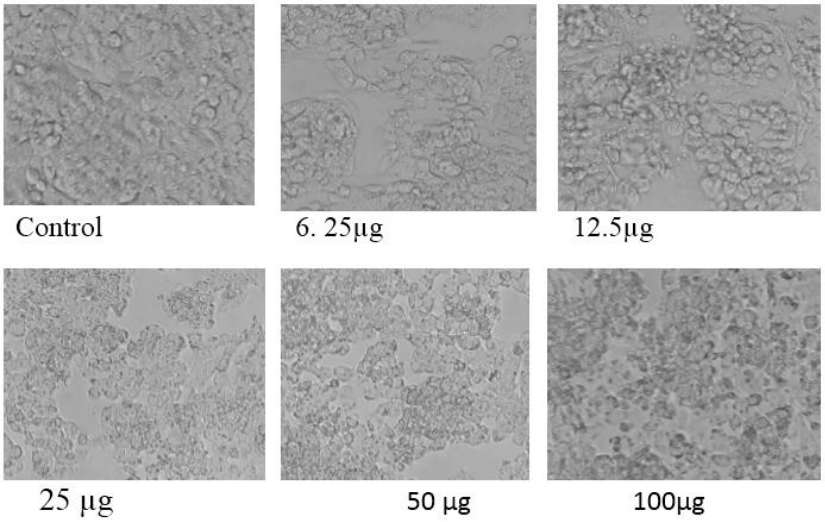


Fig 9: SAMPLE A

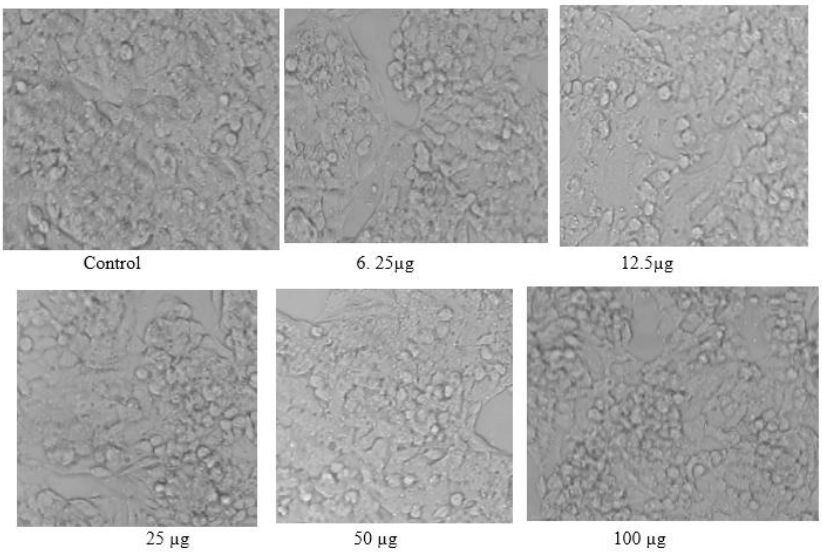


Fig 10: SAMPLE B

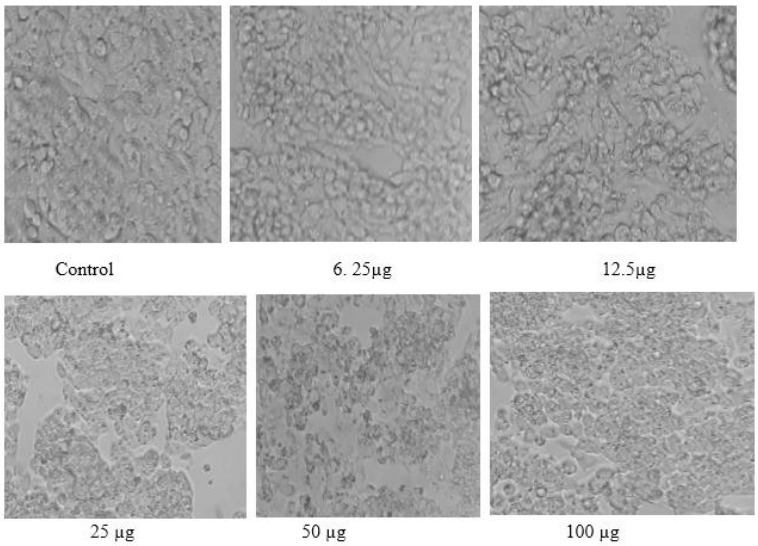


Fig 11: SAMPLE C

4. Conclusion

Berberine and quercetin are individually effective against NAFLD whereas the combination of these both has more efficacy as well as there is no chemical modification taking place. The combination shows both a decrease in NAFLD and reducing inflammation in the liver. The synergistic activity of Berberine and quercetin may be responsible for the effects on NAFLD, and it is essential that further research be conducted to understand the exact mechanisms by which they function together.

Conflict Of Interest: The authors declare no potential conflict of interest

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