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Original Article

The Effectiveness of Hydroalcoholic Extract and Fraction of *Cucumis melo* on Amelioration of High Fat Diet Induced hepatotoxicity: A Comprehensive Approach Integrating Computational and Preclinical Validation

Sanjay R. Ugare[1,*], Nayeem A. Khatib[1,*], Faizan A. Beerwalaa[1,2], Ashwini Ratnakar[3], Dhanashree Patil[4], Vishal S. Patil[1,2], Darasaguppe R. Harish[2]

[1]Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.
[2]Biology and Microbiology, ICMR- National Institute of Traditional Medicine, Belagavi, Karnataka, INDIA.
[3]Department of Pathology, Jawaharlal Nehru Medical College, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.
[4]Department of Molecular Biology, Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, INDIA.

ABSTRACT

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is most prevalent global health problem, develops due to accumulation lipids in the hepatocytes in the form of Triglycerides (TG) and Free Fatty Acids (FFA). Traditional healers recommend seeds of *Cucumis melo* Linn as a liver tonic as well as for its treatment of liver cirrhosis, however its hepatoprotective mechanism have not been explored. **Objectives:** To investigate significant molecular mechanism of *C. melo* against liver cirrhosis via *in vivo* analysis followed by in silico. **Materials and Methods:** HFD was used for the induction of hepatotoxicity in mice and Silymarin as a standard control. The physical parameters were measured throughout the study along with antioxidant, serum biomarkers and histology of liver. Further, System biology tools were used to predict the possible mechanism of action. Docking studies were carried out with modulated phytocompounds against FXR protein target. **Results:** *C. melo*. Extract and fraction ameliorated the HFD induced oxidative stress and histological changes. Additionally, improved liver biochemical parameters such as AST, ALT, ALP, serum bilirubin, total bilirubin, LDL, VLDL, TC and, TP were seen remarkable significant effects. Furthermore, System biology revealed the 16 phytocompounds of *C. melo* Linn. potentially regulate the PPAR signaling. Among them, Euphol phytocompound was predicted to interact with secreted FXR and may contribute to reduced hepatotoxicity. **Conclusion:** The results suggests *C. melo* as a promising therapeutic agent for hepatotoxicity by reducing the symptoms and stress associated with diseases condition which may be due to the regulation of multiple protein via multiple phytocompounds.

Keywords: *Cucumis melo*, High Fat Diet, *In silico*, Silymarin.

Correspondence:
Dr. Nayeem A. Khatib
Professor and Head of Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi-590010, Karnataka, INDIA.
Email: khatibnayeem@hotmail.com

Mr. Sanjay R. Ugare
Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi-590010, Karnataka, INDIA.
Email: sanjayugare@gmail.com

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INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) presents a significant worldwide health concern characterized by hepatic dysfunction and persistent dyslipidemia.[1,2] This condition results in the abnormal accumulation of fat and cholesterol within liver tissue, devoid of inflammatory processes and is driven by a complex interplay of signaling mechanisms. Metabolic disorders such as obesity, type 2 diabetes, dyslipidemia and hepatic steatosis are major precursors for the pathogenesis of the liver.[1,2] Studies suggest that mitochondrial dysfunction is a major precursor pathogenesis of the liver. As per previous reports, prolonged consumption of HFD is responsible for abnormal lipid accumulation in the form of Free Fatty Acid (FFA) and Triglyceride (TG) in the serum/liver, increasing the weight of the liver, oxidative stress liable to steatohepatitis, cirrhosis, and Hepatocellular Carcinoma. (HCC).[1,2] Cholesterol major precursor of the hepatic cells, impairment in the regulation of cholesterol level, metabolism of lipoprotein, dietary uptake, endogenous synthesis excretion,[3] elevated plasma levels of Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), Triglyceride Concentration

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(TG), decrease in plasma High-Density Lipoprotein cholesterol level (HDL-C) are highly manifested leading to dyslipidemia.

The Nuclear Receptor (NR) is the biggest family ligand-modulated of transcriptional regulators, which has 49 members in mice and 48 in humans and provides a framework for a better knowledge of liver physiology and pathobiology. Endogenous and exogenous substances such as hormones, Fatty Acids (FAs), Bile Acids (BAs), medicines, toxins and intermediary molecules in metabolism are examples of ligands for NRs.[4] These occurrences play a pivotal role in governing signal transduction pathways, both in normal physiological states and during pathological conditions.[5] Over time, Numerous steroid hormone Receptors (NRs) have been identified, including the Farnesoid X Receptor (FXR), Liver X Receptor (LXR), Pregnane X Receptor (PXR), Peroxisome Proliferator-Activated Receptor (PPAR) and Retinoid X Receptor (RXR). Prior to their discovery, the natural ligands and functions of these receptors remained elusive. However, their regulation has been extensively researched and identified to regulate lipid glucose metabolism, BA homeostasis, drug disposition, reproduction, inflammation, cell differentiation and various aspects of tissue repair including liver regeneration, fibrosis and, finally, tumour formation.[6]

The current investigation aims to evaluate the potential beneficial effects of *Cucumis melo* Linn in managing the HFD- induced hepatotoxicity in mice. *C. melo* is known for its traditional medicinal consideration in India, exhibits protection against hepatic damage, dyslipidaemia and inflammation. The seeds and fruit used traditionally for multiple purpose such as obesity, cough, flatulence, jaundice. The flesh of the fruit is a considerable source of protein, fatty acids, ascorbic acid, folic acid, carotenoids, terpenes and steroidal glycosides.[7]

MATERIALS AND METHODS

Experimental Analysis

Plant collection, authentication and extraction

The fresh fruits of *C. melo* were harvested in the Belagavi region of Karnataka. By comparing morphological traits, the Central Research Facility B.M.K. Ayurveda Mahavidyalaya, Belagavi, authenticated with accession number CRF/Auth./2020/1. Initially, Soxhlet extraction technique was used for 100 g *of C. melo* seeds with solvent as 95% v/v ethanol and the obtained extract was subjected to bio-guided fractionation as shown in Figure 1.

DPPH free radical scavenging activity

The DPPH assay of *C. melo* extract and Ascorbic acid was performed as per the method of Tubachi *et al.*,[8] and the IC_{50} was determined by,

$$\% \text{ Inhibition } (IC_{50}) = \frac{\text{Abs of Control} - \text{Abs of Sample}}{\text{Abs of Control}} \times 100$$

In vitro cytotoxicity assay

HepG2 cells were seeded in 96-well plates at a density of 20000 cells/well and were incubated at 37°C for 24 hr with 95% humidity in a CO_2 incubator. After 24 hr, the cells were treated with test substance (*C. melo* extract and fraction), standard (Silymarin) and HFD and was incubated at 37°C and 5% CO_2. Cytotoxicity was evaluated after 24 hr by adding 20 µL of MTT solution (5 mg/mL in phosphate buffer saline) for 4 hr incubation followed by 100 µL DMSO for dissolving formazan crystals. The intensity of color was measured at 570 nm using an ELISA plate reader and CC_{50} was determined.[9]

Animal studies

Animal selection and ethical approval

Healthy Swiss albino mice (15-20 g) were purchased from Shri Venkateshwara Enterprises. The ethical clearance was obtained at KLE College of Pharmacy, Belagavi IAEC (Reg No. 221/Po/Re/S/2000/CPCSEA).

Preparation of High-Fat Diet (HFD) and model for hepatoprotective activity of *C. melo*

High fat diet was prepared by mixing the powder pallet diet+5g of Ghee (34%)+1 g of Vanaspati (18%)+5 g of yeast powder (8%)+5 g of lard (12%)+1 mL of Coconut oil (6%), totally 78% of calories from fat and make up the volume up to 10 mL/kg. The percentage of fat is conformed according to the RM value.[10] Albino mice were acclimatized for 7 days, then divided into six groups (n=6). Except for group I, all group animals received HFD daily for 28 days while Silymarin (50 mg/kg p.o) served as the standard. Group I: food and water. Group II: HFD (10 mL/kg).[11] Group III: Silymarin 50 mg/kg and HFD (10 mL/kg). Group IV: 50mg/kg of C. melo fraction and HFD (10 mL/kg), and lastly Group V: 500mg/kg of *C. melo* extract and HFD (10 mL/kg). 24 hr after the last dosing, a blood sample was collected through the retro-orbital puncture and animals were sacrificed by cervical dislocation under general anaesthesia. The liver was collected for estimation of biochemical parameters and liver histopathological examination.

In silico analysis

Collection of phytocompounds

Phytocompounds of *C. melo*. were retrieved from reported literature and open database viz., Dr. Dukes DB (https://www.nal.usda.gov/dr-dukes-database), IMMPAT DB (https://cb.imsc.res.in/imppat/), Phytochemical integration DB (https://www.genome.jp/db/pcidb/) and their structures with PubChem CID and SMILES were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Druggability and Toxicity analysis

Molsoft tool was used to predict the Druglikeness score for each phytocompound based upon Lipinski's rule of 5. Moreover, Molecular Weight (MW), Formula (MF), number of Hydrogen Bond Acceptors (HBA), Donor (HBD). Surface area (MolPSA) and lipophilicity (MolLogP) of screened phytocompounds were obtained. The adverPred database[12] (https://www.way2drug.com/adverpred/) was used to predict the side effects for the screened phytocompounds.

ADME profiling

We used ADMETlab 2.0 (https://admetmesh.scbdd.com/)[13] to predict the ADME of the selected phytocompounds which is been widely recognized for their pharmacokinetics properties i.e., absorption, distribution, metabolism, and excretion.

Target prediction

SuperPred (https://prediction.charite.de/subpages/targetprediction.php)[14] server, was used to predict targets of each phytocompounds with a *p*-value of ≥0.7 and their respective Gene ID were obtained from UniProtKB server (https://www.uniprot.org/).

Enrichment analysis and network construction

A set of predicted protein targets modulated by the phytocompounds targeting FXR were submitted to STRING[15] ver 11.0 (https://stringdb.org/) and were used to identify the modulated pathways by a set of queried gene targets. Additionally, KEGG,[16] genomes pathway database and published literature were also used to compile modulated genes and their related pathways in liver cirrhosis. Finally, using Cytoscape[17] ver 3.6.1 a network was created between phytocompounds, targets and pathways using edge count as a topological parameter. For the network representation, the map node was set from low to high and small to big.

Molecular docking

3D structure of each phytocompounds and known standard inhibitor Silymarin were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in "sdf" file format and converted into "pdb" format using Discovery Studio Visualizer (https://discover.3ds.com/discoverstudio-visualizer-download) ver 17.2. Similarly, FXR (PDB Id: 6HL1) was retrieved from Protein Data Bank[18] (RCSB; www.rcsb.org). Molecular docking was performed using AutoDock Vina via executed through the POAP pipeline.[19] Standard docking was performed and the grid was set around the active site with the box dimensions center x=9.070, y=17.169, z=13.316 and size x=27.408, y=29.430 and z=23.349 and exhaustiveness was set to 50. The binding results were further, analyzed for interaction between ligand and target and were visualized using 'BIOVIA Discovery studio visualizer 2019'.

Statistical analysis

All the data were expressed as mean ± SEM. The data was analyzed by One way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Prism version 8 with $p<0.05$ considered statistically significant and linear regression was used to analyze the inhibitory constant. Enrichment analyses were analyzed using the whole genome and edge count was defined to analyze the network representation, kcal/mol showed the least binding energy.

RESULTS

Experimental analysis

Extract yield and preliminary phytochemical and physiochemical analysis

The hydroalcoholic extract yield of *C. melo*. seeds per 100 g w/w of powder was found to be 16%. Additionally, the *C. melo* fraction yield for 16 g w/w of hydroalcoholic extract was calculated as 6% respectively.

In vitro antioxidant assay

The IC_{50} of ascorbic acid and *C. melo* was found to be 47.89 and 153.0 µg/mL, respectively as shown in (Figure 2).

In vitro cytotoxicity assay in HepG2 cell line

C. melo extract and its fraction (Conc. 12.5-100 µg/mL) showed the significant cell viability after 24 hr (Figure 3).

In vivo studies

Effect of C. melo on body weight and liver weight

Disease control group (HFD) exhibited remarkable raise in body weight and liver weight compared to normal group ($p<0.001$). Positive control groups, *C. melo* fraction and extract group exhibited decline in body weight ($p<0.001$) and liver weight ($p<0.001$) compared to disease control group (Figure 4).

Effect of *C. melo* on antioxidant markers

Disease control group (HFD) exhibited remarkable decrease ($p<0.001$) in Catalase, Nitrate, SOD and GSH level and increase in the LPO level compared to normal group. Whereas, treatment with Silymarin, *C. melo* fraction and *C. melo* extract reversed ($p<0.001$) the same compared to disease control group. Among these, *C. melo* fraction exhibited potent antioxidant property (Figure 5).

Effect of *C. melo* on liver function

Disease control group (HFD) exhibited remarkable increase ($p<0.001$) in ALP, ALT, AST, total and serum bilirubin compared to normal group. Whereas, treatment with Silymarin, *C. melo* fraction and *C. melo* extract showed significant decrease ($p<0.001$) in the liver enzymes compared to disease control group. Among

these, *C. melo* fraction was found to be good hepatoprotective compared to extract (Figure 6).

Effect of *C. melo* on lipid profile

Disease control group (HFD) exhibited remarkable increase ($p<0.001$) in LDL, VLDL, TC, TG and decrease ($p<0.001$) in the HDL and TP compared to normal group. Whereas, treatment with Silymarin, *C. melo* fraction and *C. melo* extract showed significant reverse in the parameters ($p<0.001$) compared to disease control group (Figure 7).

Histological examination

In mice receiving vehicle, liver tissue containing a Central Vein (CV) appeared unharmed. Histological abnormalities that suggested liver cirrhosis showed clogged blood vessels, disintegrating tissue, fatty degeneration and excessive inflammation were improved after HFD treatment. Pretreatment with Silymarin, extract and fraction of *C. melo* anomalies was brought back to normal as shown in Figure 8.

In silico analysis

Mining of phytocompounds from C. melo

A total of 160 phytocompounds were reported to be present in the *C. melo*.

Draggability and druglikeness profile

Out of 160 phytocompounds, 16 phytocompounds were predicted to have a positive drug likeness score in which 24-methylenecyloartanol showed the highest molecular weight with 1 HBD and HBA of each, followed by Euphol with 426.7,

Table 1: **Binding energy of ligands with FXR protein.**

Compounds	BE kcal/mol	HBI	NHBI	Total number of residues	Number of interactions with active sites
* Silymarin	-9.4	Arg331…O, Arg331…OH, Tyr369…O	Met290, Ile352, Tyr369, Met265, Ile335	8	6
Euphol	-11.1	Tyr361…O, His447…O	Trp454, Ala291, His294, Met265 (3), Arg264, Val297, Met290 (3), Met328 (4), Leu348 (2), Leu287, Ile352 (2), Phe329, Met450, Tyr361 (2)	26	12

*Standard compound; BE: Binding energy; HBI: Hydrogen bond interaction (s); NHBI: Non- hydrogen bond interaction (s).

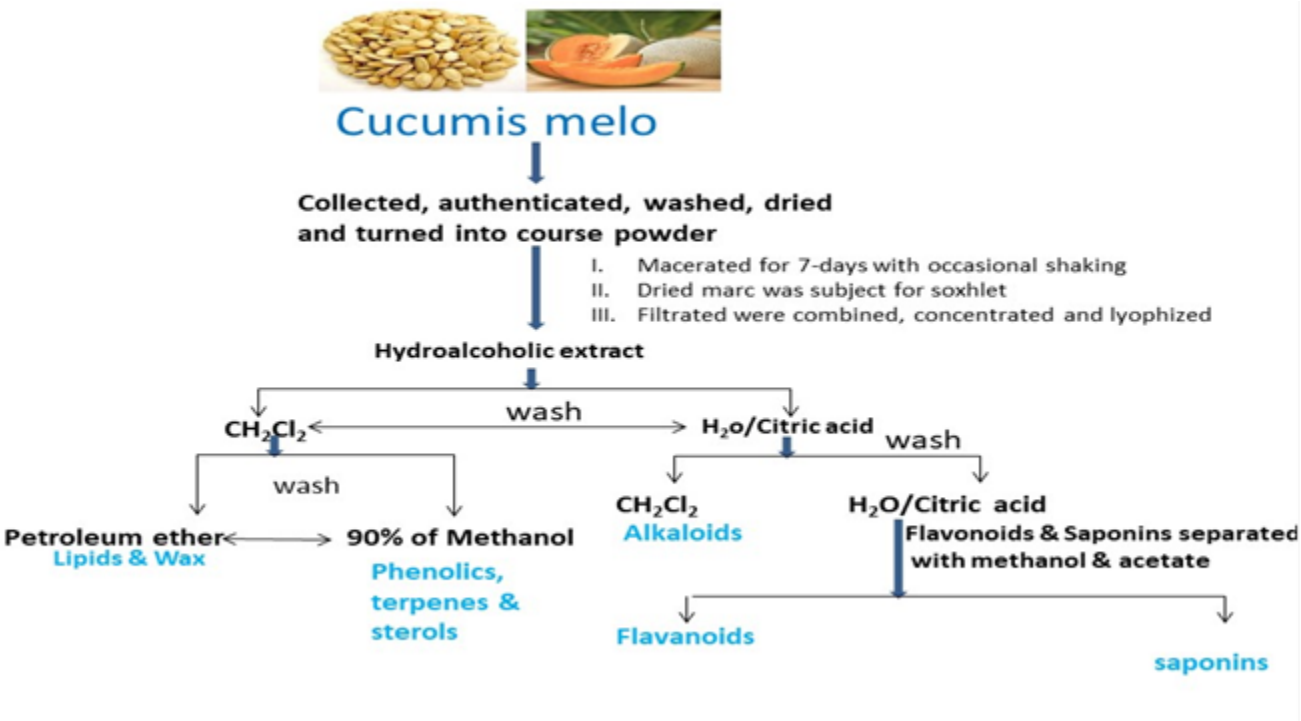

Figure 1: Hydroalcoholic extraction and crude fractionation of *Cucumis melo.*

the least was observed in Avenasterol and Stigmasterol with molecular weight 412.7. Moreover, none of the phytocompounds were identified to cross the blood-brain barrier as shown in Table S1.

Toxicity screening and ADME profiling

Among all the screened phytocompounds (as shown in Table S2 and Table S3), Avenasterol and Sitosterol were predicted to have toxicity, but were observed within the limits of Pa 0.246 and Pi 0.228.

Gene enrichment analysis and network construction

Gene enrichment analysis showed that 16 phytocompounds were predicted to modulate the 27 targets. Additionally, these 27 targets interacted with each other to modulate the 65 pathways in the KEGG database. Among, them 7 pathways were identified to be associated with liver cirrhosis. As depicted in Table S4 and Table S5, the PPAR signaling pathway scored the lowest FDR of 1.95E-13 by modulating the 8 protein targets, followed by insulin resistance and AMPK pathway. Furthermore, out of 16 phytocompounds, Euphol was found to highly modulated compound by regulating a maximum number of proteins (Figure 9).

Molecular docking

The active site residues of *FXR*, namely *Ser259, Tyr260, Gln263, Arg264, Met265, Thr270, Ile273, Leu287, Thr288, Met290, Ala291, His294, Val297, Leu298, Phe301, Asn327, Met328, Arg331, Ser332, Ile335, Phe336, Lys338, Ser345, Leu348, Ile352, Ile357, Tyr361, Ile362, Met365, Phe366, Tyr369, Thr386, His447, Met450, Trp454 and Trp469* were taken from the crystal structure "6HL1.pdb". Among all the phytocompounds, Silymarin scored the lowest Binding Energy (BE) of -9.4kcal/mol via forming 3 hydrogen bonds and 5 non- hydrophobic interactions (Figure 10). Euphol scored the lowest binding energy among all others

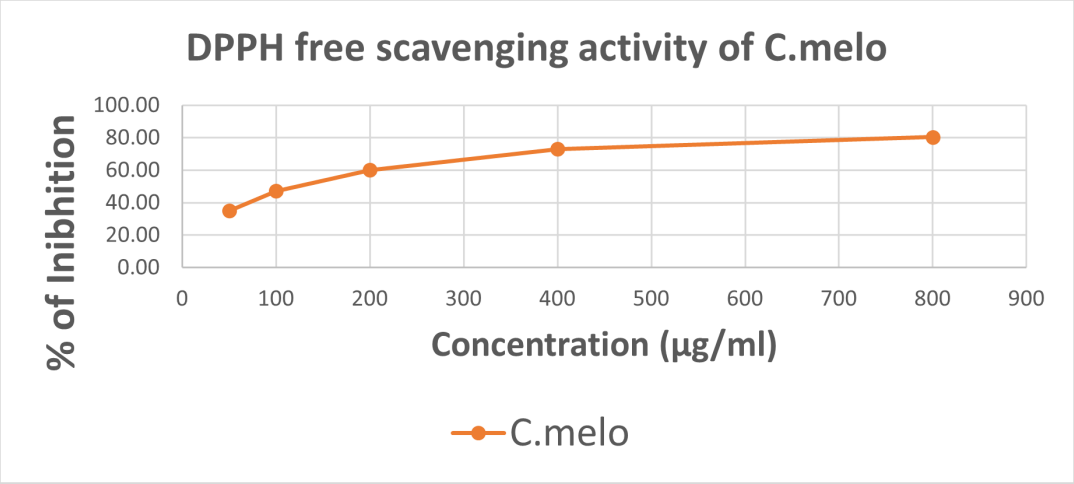

Figure 2: Antioxidant activity *C. melo* by DPPH assay.

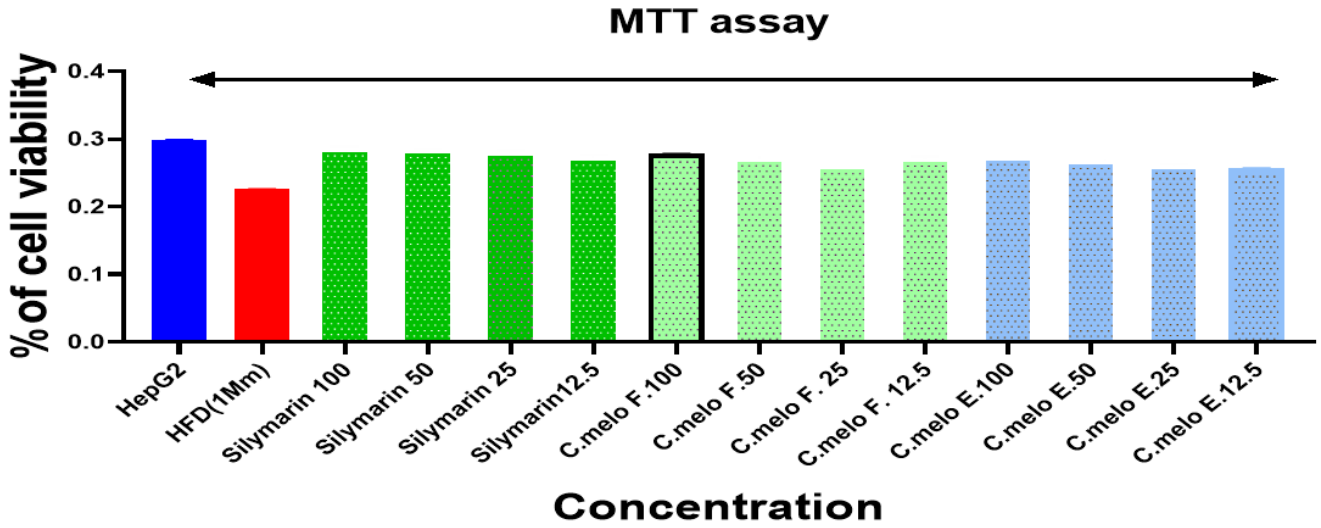

Figure 3: MTT cytotoxicity activity.

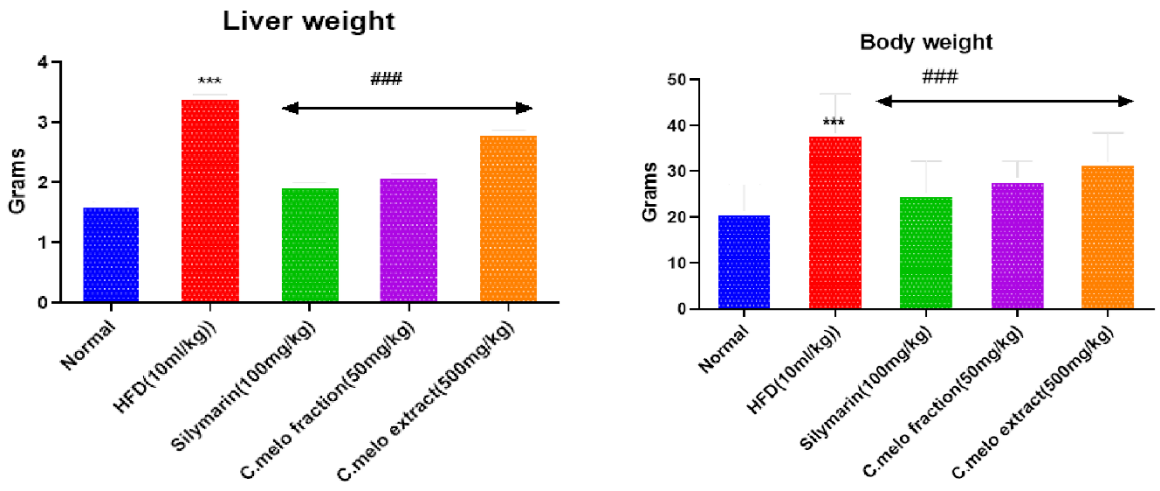

Figure 4: Effect of *Cucumis melo* extract and fraction on body weight and liver weight. ***p<0.001 compare to normal, ###p<0.001 when compared to disease control.

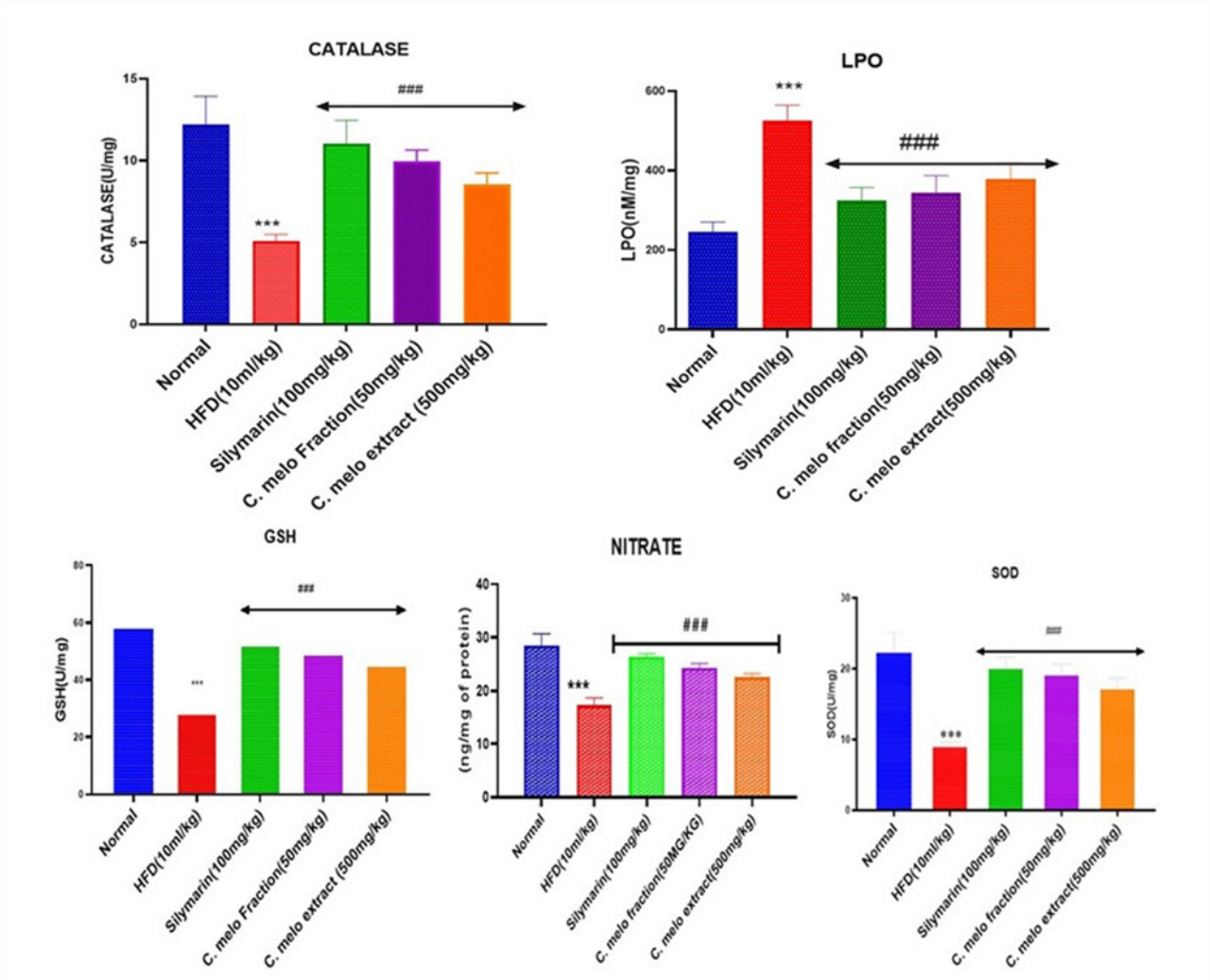

Figure 5: Effect of *Cucumis melo* extract and fraction on catalase, GSH, Nitrate and LPO. ***p<0.001 compare to normal, ##p<0.01, ###p<0.001 when compared to disease control.

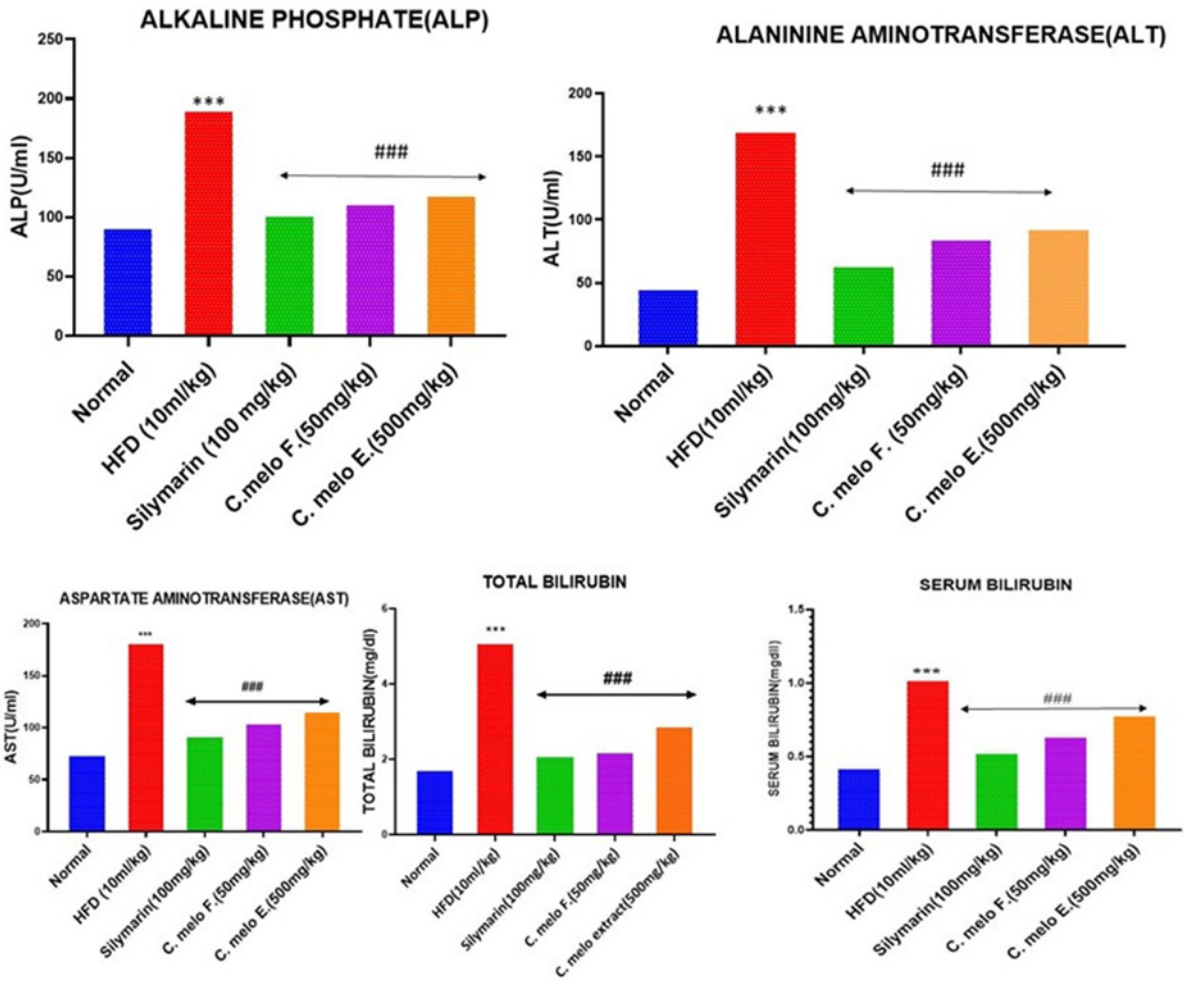

Figure 6: Effect of *Cucumis melo* extract and fraction on AST, ALT, ALP, Serum bilirubin and Total bilirubin. ***p<0.001 compare to normal, ##p<0.01, ###p<0.001 when compared to disease control.

with a BE of -11.1kcal/mol *via* forming 2 hydrogen bonds and 23 non-hydrophobic interactions (Figure 11 and Table 1).

DISCUSSION

The present study was designed to explore the hepatoprotective and antioxidant effect of *C. melo* in HFD-induced hepatotoxicity in albino mice. In HFD animals, elevated hepatic fatty acid metabolism results in cholesterol and triglyceride accumulation as well as elevated liver inflammation and liver damage.[20] Firstly, both *C. melo* extract and fraction showed significant cell viability compared to HFD-treated groups in HepG2 cells. Secondly, the extract and a fraction were screened for its hepatoprotection in albino mice. In animal study, the significantly increased body weight (p<0.001) and liver weight (p<0.001) could be due to the accumulation of fat which was reversed in the treatment groups. This indicates the potential role of phytosterols in the regulation of lipid metabolism. In the present study, HFD-treated animals showed abnormal levels of antioxidant markers TP, GSH, LPO, SOD, nitrate and catalase, levels and were reversed in the *C. melo* extract and fraction groups. Similarly, lipid markers like HDL, LDL, VLDL, TG and TC were also abnormal in the HFD-treated animal and were found to be towards normal in *C. melo* extract and fraction groups. Serum ALP, AST, ALT and bilirubin are considered as a specific marker for hepatic damage and were significantly elevated in the HFD-treated animals and were found to be towards normal *in C. melo* extract and fraction groups. Histology of the liver showed remarkable histological changes after treatment. In HFD-treated groups, cirrhosis or liver injury was confirmed by clogged blood vessels, disintegrating tissue, fatty degeneration and excessive inflammation. Whereas, Silymarin, *C. melo* extract and fraction anomalies HFD diet mediated histological pattern of the liver.

Moreover, the current work traced the 160 reported phytocompounds of *C. melo* to propose a probable mechanism against the HFD-induced hepatotoxicity. Out of 160 phytocompounds, 16 were predicted to show a positive druglikeness and were further predicted to target 27 protein molecules and were involved in 65 pathways, in which 7 pathways

were identified to be associated with liver cirrhosis. Among 27 predicted targets FXR, HMGCR was identified as a major modulated and therapeutic target involved in the pathogenesis of hepatotoxicity by modulating all 7 pathways within the network. FXR indirectly reduces lipid-induced hepatic inflammation by decreasing intracellular FA levels.[21] It is highly expressed in the liver and intestine which further standalone in activating varieties of inflammatory markers causing hepatocellular or cholestatic conditions.[21] Several tetracyclic triterpenes from fruits and vegetables have been reported to inhibit the production of FXR by blocking the inflammatory cellular signaling pathways primally MAPK, PI3-Akt and NF-kB.[22] *C. melo* phytocompounds exhibit potent antioxidant, anti-inflammatory and anti-colitis activity by reducing the FXR expression in bile formation,

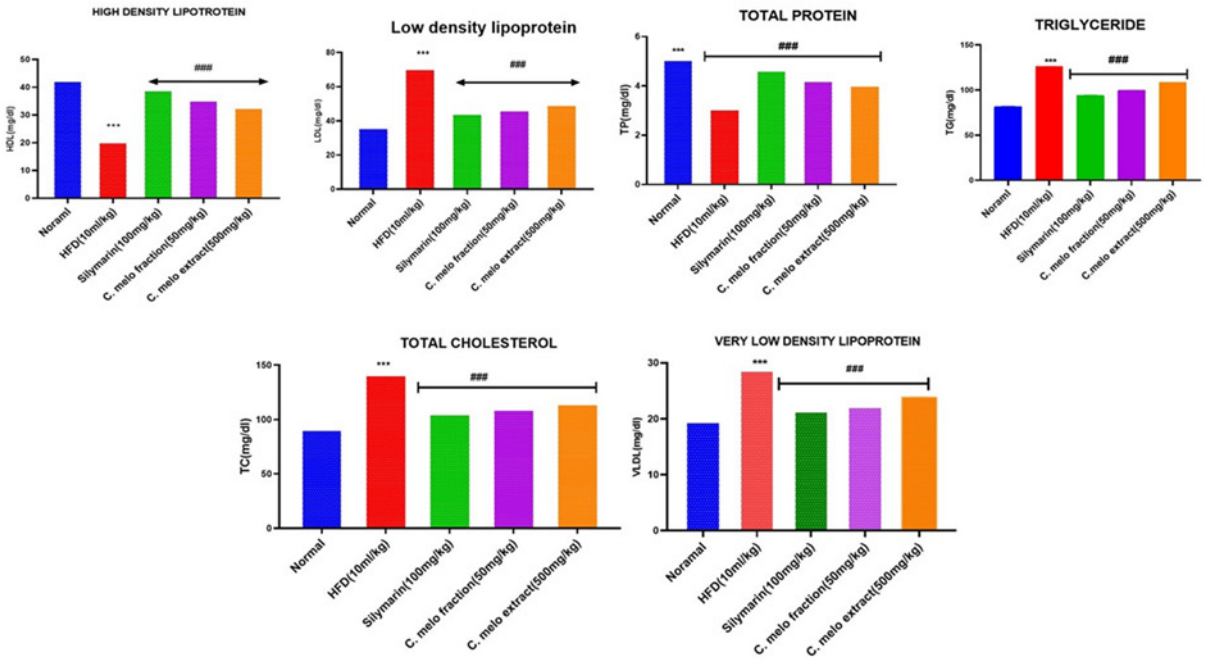

Figure 7: Effect of *Cucumis melo* extract and fraction on HDL, LDL, VLDL, TC, TG, TP. ***$p<0.001$ compare to normal, ###$p<0.001$ when compared to disease control.

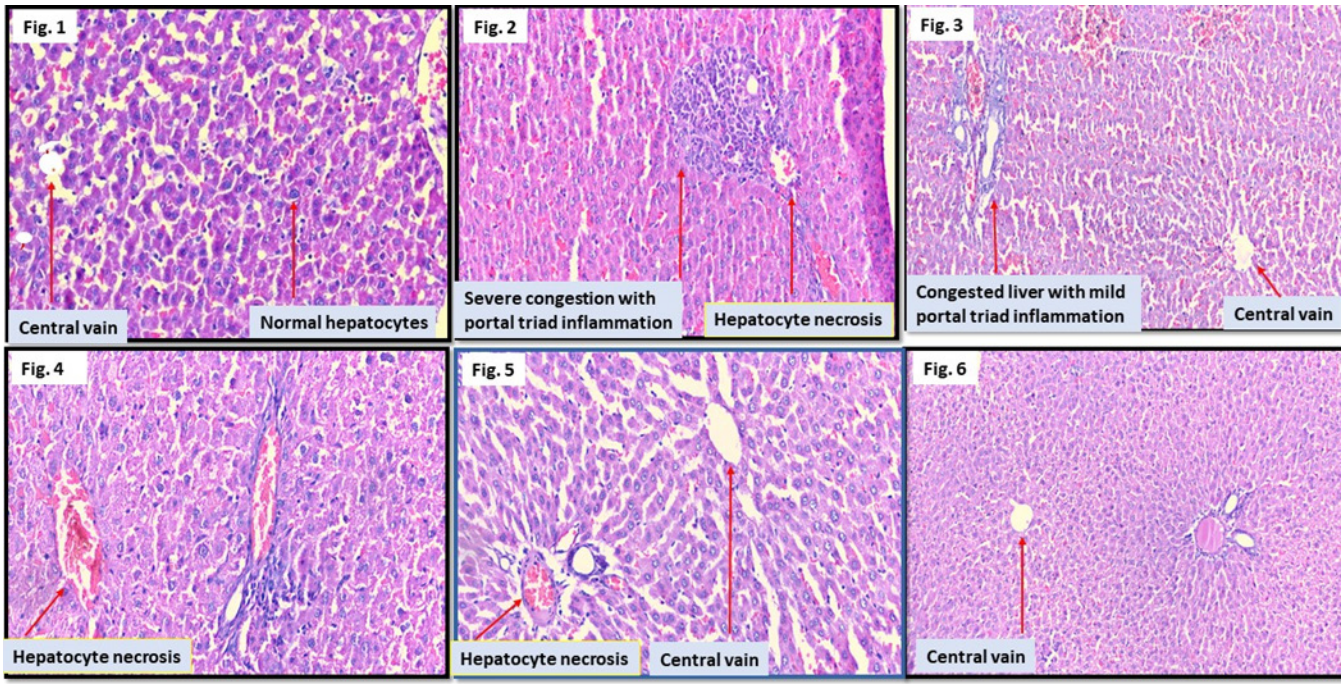

Figure 8: Histopathological changes on HFD induced hepatotoxicity. I: Normal control, II: HFD, III: Silymarin+HFD, IV: *C. melo* extract [250 mg/kg+HFD], V: *C. melo* extract [500 mg/kg+HFD], VI: *C. melo* fraction [50 mg/kg+HFD].

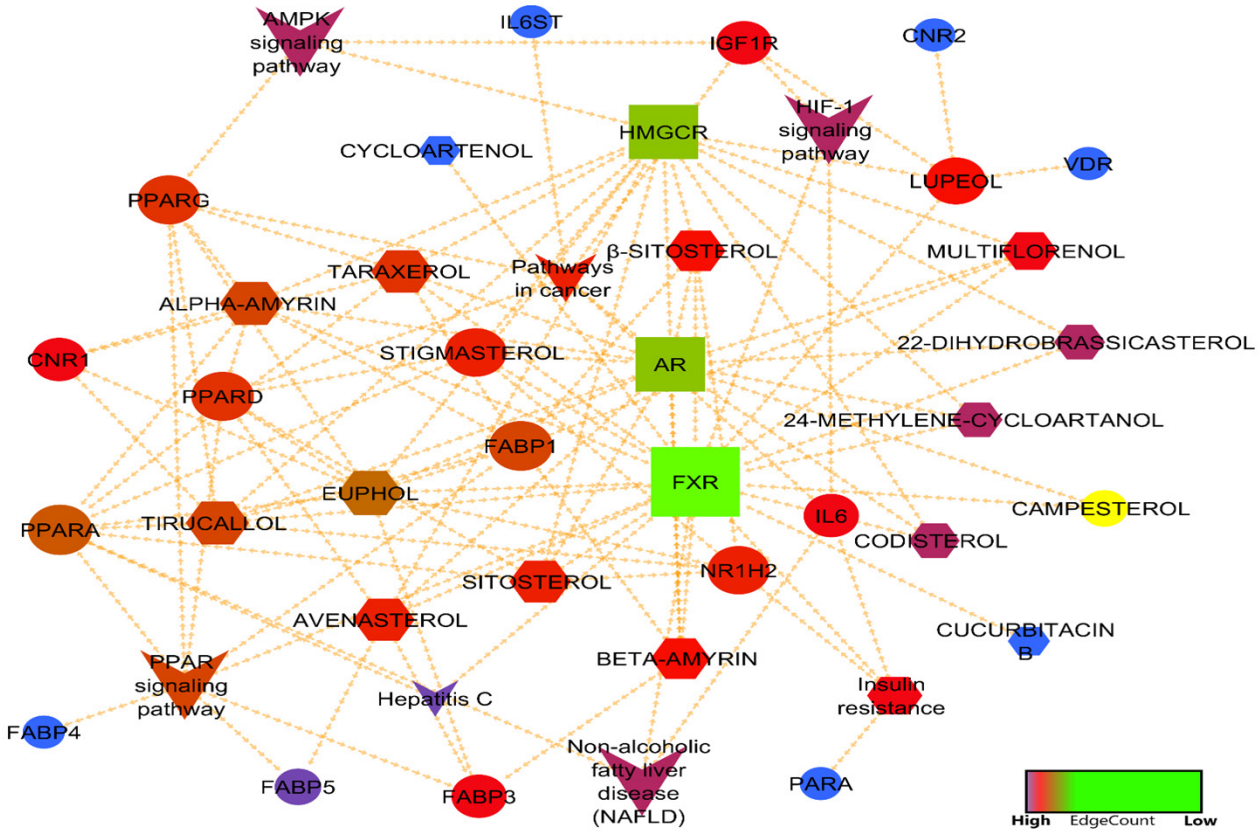

Figure 9: Network representation of *Cucumis melo* Linn phytocompounds with their probable protein molecules involved in Liver cirrhosis.

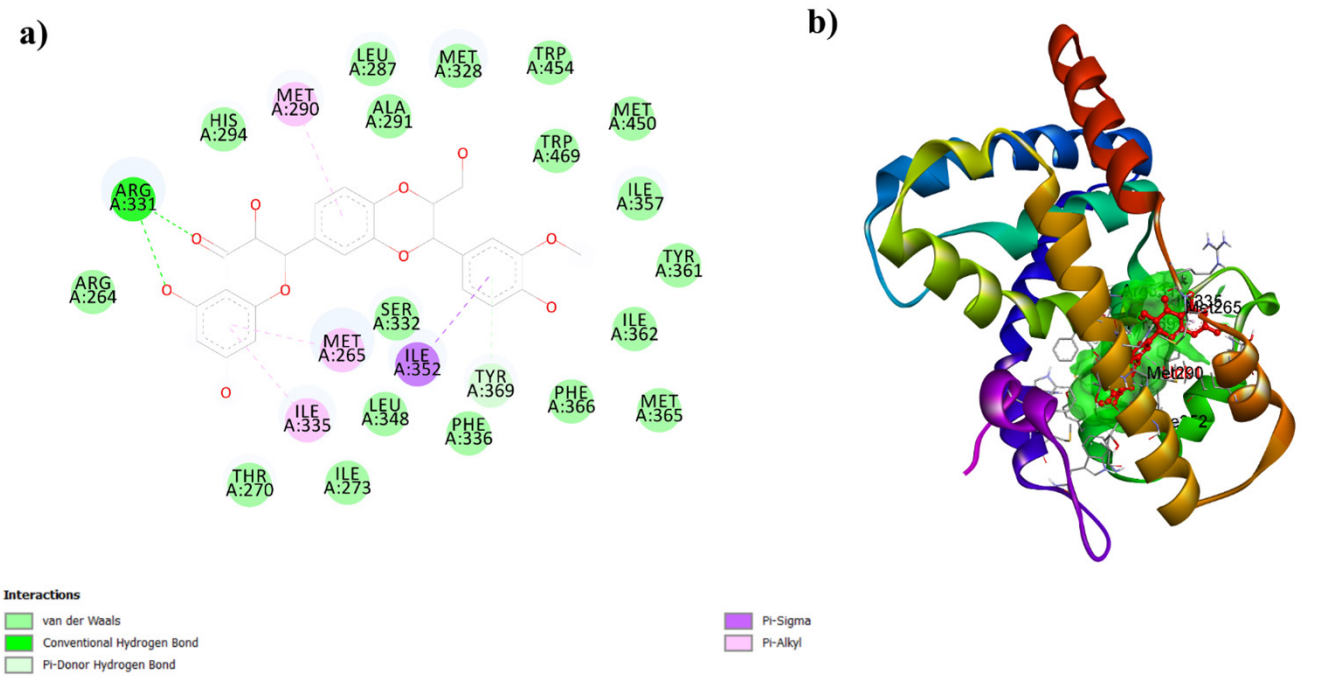

Figure 10: Shows (a) the 2D interaction of the binding mode of silymarin with FXR, (b) 3D binding mode of silymarin with FXR.

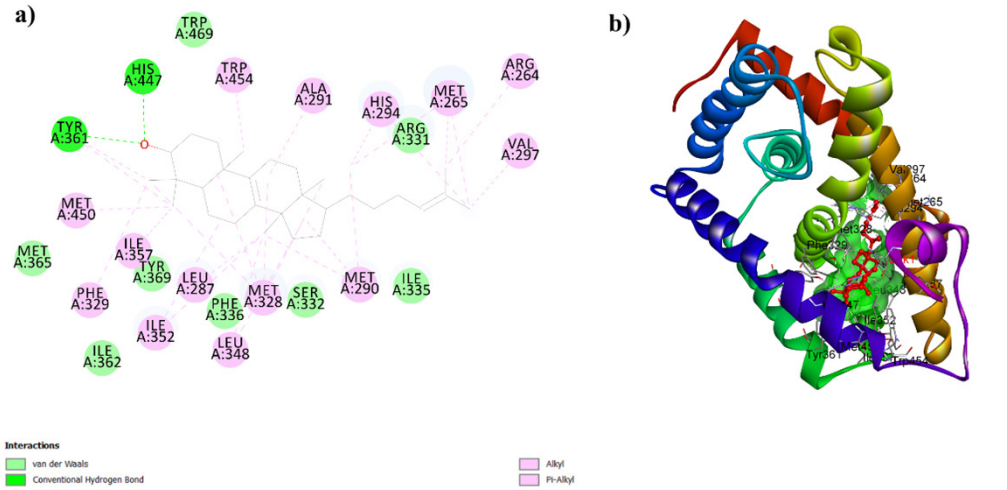

Figure 11: Shows (a) the 2D interaction of the binding mode of euphol with FXR, (b) 3D binding mode of euphol with FXR.

TNF-α and IL-6 upregulations.[23] Furthermore, *in vitro* and *in vivo* studies demonstrated that euphol showed a both preventive treatments were effective in reducing the severity of inflammatory conditions and reducing intestinal smooth muscle with propulsion of faces by controlled acute inflammatory reactions. Also, it regulates nitric oxide production and mucosal damage and decreases in the component expression of inflammatory responses.[24] Therefore, the network analysis and predicted affinity of phytocompounds of *C. melo*. towards the active site residues of FXR seems to be concurrence with these findings and provide the possible molecular mechanism of action of *C. melo*. as a potent hepato-protective nutraceutical.

In network, non-alcoholic fatty liver diseases, AMPK, HIF-1, Insulin resistance, PPAR signalling and pathways in cancer were found to be highly modulated pathways via modulating multiple protein molecules. On looking into the Non-alcoholic fatty liver diseases pathway PPAR signaling pathway it includes 9 potential targets i.e., FABP4, PPARG, FABP1, FABP5, PPARD, FABP3, PPARA and, FXR. The current study identifies the tetracyclic triterpene and triterpenoids to majorly target PPAR signalling pathway by targeting the 9 protein targets, among which FXR is also present. Activation of the PPAR signalling pathway modulates lipid metabolism and energy homeostasis.[25,26] In some conditions the impaired activity of PPAR signalling pathway might contribute to Hepatocellular carcinoma.[27] Insulin resistance mechanism is a vital signaling pathway in which it binds to the FXR which further extends its role in regulating lipid and cholesterol homeostasis. Activation of FXR reverses the insulin resistance and lipid abnormalities gaining its protection against liver toxicity.[28] Further, it is well reported that, phytocompounds from *C. melo* act as an anti-inflammatory, anti-diabetic, diuretic, gastroprotective, anti-microbial and, hepatoprotective activity by inducing the antioxidant effect. Also, phytocompounds present in the *C. melo* are reported to target genes involved in hepatotoxicity. Hence, the present investigation on hepato-protective activity of *C. melo* could be due to the modulation of PPAR, Insulin resistance, pathways in cancer, AMPK, non-alcoholic fatty liver disease and HIF-1 signaling pathways.

CONCLUSION

The current investigation employed *in vivo* experimental evaluation to explore the HFD-induced hepatoxicity of *C. melo* extract and fraction followed by computational approaches. Evaluation of the extract and fraction demonstrated an increase in antioxidant enzyme activity and reduced biochemical levels. Histopathology analysis revealed improved clogged vessels, disintegrating tissue, fatty degeneration and excessive inflammation. Further, we depicted the interaction of the phytocompounds from *C. melo*. with proteins involved in the etiology of hepatotoxicity. Gene enrichment analysis identified PPAR signalling pathway as a likely major pathway targeted by the phytocompounds to counter hepatotoxic activity. Key constituent like euphol suggested their potential role in inhibiting the FXR target. Further, the identified phytocompounds and enriched fractions need to be validated for clinical trials and their interaction studies with the standard anti-hepatotoxicity drugs to develop and formulate the adjunct therapies for effective management. On the other hand, it provides the critical suggestive possible mechanism of action that may be taken advantage of developing the *C. melo*. based research plans.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BE: Binding energy; ***C. melo:*** *Cucumis melo*; **FDR:** False Discovery Rate; **FFA:** Free Fatty Acid; **FXR:** Farnesoid X Receptor; **HBI:** Hydrogen bond interaction (s); **HCC:** Hepatocellular Carcinoma; **HFD:** High Fat Diet; **NAFLD:** NHBI: Non-hydrogen bond interaction (s); Non-Alcoholic Fatty Liver Disease.

SUPPLEMENTARY DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

CONTRIBUTIONS

SRU: Conceptualized the research, Experimentation and wrote the original draft. NAK: Conceptualized the research, revised the draft and supervision. FB, AR and DP: Assisted SRU in experimentation and writing manuscript. VSP: Revision and drafting. HRD: Conceptualized the research and revision. All authors have reviewed and approved the final version of the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets analyzed during the current study are available from Sanjay R. Ugare (First Author) and Nayeem A. Khatib (corresponding author) on request.

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Table S1: Phytocompounds of *C. melo* which were retrieved from PubChem possess Drug likeness score.

Sl. No.	Compounds	Lipinski Rules				
		Molecular wt.	HBA	HBD	Log P	Drug likness score
		(G/mol)				
	Acceptable Values	<500	<5	<5	<10	0 to 2
1.	Euphol	426.7	1	1	8.74	0.55
2.	Tirucallol	426.7			8.74	0.55
3.	Alpha-amyrin	426.7	1	1	7.77	0.10
4.	Taraxerol	426.7	1	1	8.11	-0.9
5.	Avenasterol	412.7	2	1	7.95	0.25
6.	Stigmasterol	412.7	1	1	7.74	0.62
7.	Sitosterol	414.7	1	1	8.45	0.78
8.	Lupeol	426.39	1	1	8.35	-0.22
9.	beta-Amyrin	426.7	1	1	7.95	-0.22
10.	beta-Sitosterol	414.39	1	1	8.45	0.78
11.	Multiflorenol	426.39	1	1	7.95	-0.27
12.	Codisterol	398.7	1	1	7.7	0.43
13.	22-dihydrobrassicasterol	400.7	1	1	7.87	0.59
14.	24-Methylenecycloartanol	440.7	1	1	8.49	-0.48
15.	Campesterol	400.37	1	1	7.87	0.59
16.	Cycloartenol	426.7	1	1	8.56	-0.27

Table S2: Toxicity screening of phytocompounds from *Cucumis melo*.

Sl. No.	*Cucumis melo*	Pa	Pi	Side Effect
	Euphol	-	-	-
	Tirucallol	-	-	-
	Alpha-amyrin	-	-	-
	Avenasterol	0.246	0.228	Nephrotoxicity
	Sitosterol	0.258	0.205	Nephrotoxicity
	Taraxerol	-	-	-
	beta Amyrin	-	-	-
	beta-Sitosterol	-	-	-
	Multiflorenol	-	-	-
	Codisterol	-	-	-
	22-dihydrobrassicasterol	-	-	-
	24-Methylenecycloartanol	-	-	-
	Campesterol	-	-	-
	Cycloartenol	-	-	-
	beta-Sitosterol	-	-	-
	Multiflorenol			

Pa: Probable activity to cause toxicity.
Pi: Probable inactivity to cause nontoxicity.

Table S3: ADME profile of each phytocompounds from *Cucumis melo.*

Compounds	GI Absorption	BBB permeability	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	LogKp (skin permeation)
Euphol	Low	No	No	No	No	No	No	-2.58
Tirucallol	Low	No	No	No	No	No	No	-2.58
Alpha-amyrin	Low	No	No	No	No	No	No	-2.51
Taraxerol	Low	No	No	No	No	No	No	-2.3
Avenasterol	Low	No	No	No	No	No	No	-2.72
Stigmasterol	Low	No	No	No	Yes	No	No	-2.74
Sitosterol	Low	No	No	No	No	No	No	-2.2
Lupeol	Low	No	No	No	No	No	No	-1.9
beta-Amyrin	Low	No	No	No	No	No	No	-2.41
beta-Sitosterol	Low	No	No	No	No	No	No	-2.2
Multiflorenol	Low	No	No	No	No	No	No	-2.41
Codisterol	Low	No	No	No	Yes	No	No	-2.53
22-dihydrobrassicasterol	Low	No	No	No	No	No	No	-2.5
24-Methylenecycloartanol	Low	No	No	No	No	No	No	-1.67
Campesterol	Low	No	No	No	No	No	No	-2.5
Cycloartenol	Low	No	No	No	No	No	No	-1.96

Table S4: Liver cirrhosis pathway and gene enrichment analysis.

Pathway	Description	Count Fin Gene Set	False Discovery Rate	Genes
hsa03320	PPAR signaling pathway.	8	1.95E-13	FABP4, PPARG, FABP1, FABP5, PPARD, FABP3, PPARA, FXR.
hsa04931	Insulin resistance.	4	0.00012	NR1H2, PPARA, IL6, FXR.
hsa04152	AMPK signaling pathway.	3	0.0039	IGF1R, PPARG, HMGCR, FXR.
hsa05200	Pathways in cancer.	6	0.00014	IGF1R, PPARG, PPARD, AR, IL6ST, IL6.
hsa04932	Non-Alcoholic Fatty Liver Disease (NAFLD).	3	0.0058	PPARA, IL6, FXR.
hsa05160	Hepatitis C	2	0.042	PPARA, FXR.

Table S5: Network based gene set enrichment analysis highlights different proteins and pathway in pathogenesis of liver.

Sl. No.	Pathway	Proteins
1.	PPAR signalling pathway.	(Fatty acid binding protein adipocyte) FABP4.
2.	PPAR signalling pathway.	Fatty acid-binding protein, liver (FABP1).
3.	PPAR signalling pathway.	Fatty acid binding protein muscle (FABP3).
4.	PPAR signalling pathway.	Fatty acid binding protein skin (FABP5).
5.	PPAR signalling pathway.	Peroxisome proliferator-activated receptor alpha (PPARA).
6.	PPAR signalling pathway.	Peroxisome proliferator-activated receptor delta (PPARD).
7.	PPAR signalling pathway.	Peroxisome proliferator-activated receptor gama (PPARG).
8.	PPAR signalling pathway.	Farnesoid X receptor (FXR).
9.	Pathways in cancer.	Peroxisome proliferator-activated receptor gama (PPARG).
10.	Pathways in cancer.	Peroxisome proliferator-activated receptor delta (PPARD).
11.	Pathways in cancer.	Androgen receptor (AR).
12.	Pathways in cancer.	Interleukin-6 (IL6).
13.	Pathways in cancer.	Insulin like growth factor1 receptor (IGF1R)
14.	Pathways in cancer.	Interleukin 6 cytokine family signal transduced(IL6ST).
15.	Insulin resistance.	Nuclear Receptor Subfamily 1 Group H Member 2(NR1H2).
16.	Insulin resistance.	Peroxisome proliferator-activated receptor alpha (PPARA).
17.	Insulin resistance.	Interleukin-6 (IL6).
18.	Insulin resistance.	Farnesoid X receptor (FXR).
19.	Non-alcoholic fatty liver disease.	Peroxisome proliferator-activated receptor alpha (PPARA).
20.	Non-alcoholic fatty liver disease.	Interleukin-6 (IL6).
21.	Non-alcoholic fatty liver disease.	Nuclear Receptor Subfamily 1 Group H Member 3(NR1H3).
22.	AMPK signalling pathway.	Peroxisome proliferator-activated receptor gama (PPARG).
23.	AMPK signalling pathway.	Insulin like growth factor1 receptor (IGF1R).
24.	AMPK signalling pathway.	HMG-CoA reductase (HMGCR).
25.	AMPK signalling pathway.	Farnesoid X receptor (FXR).
26.	Hepatitis C	Peroxisome proliferator-activated receptor alpha (PPARA).
27.	Hepatitis C	Farnesoid X receptor (FXR).
28.	HIF1 Signalling pathway	Insulin like growth factor1 receptor (IGF1R)
29.	HIF1 Signalling pathway	Interleukin-6 (IL6).