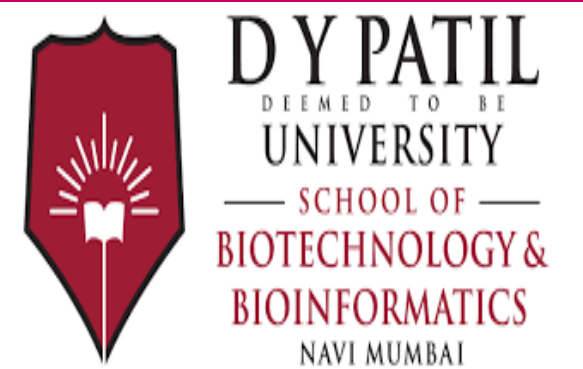
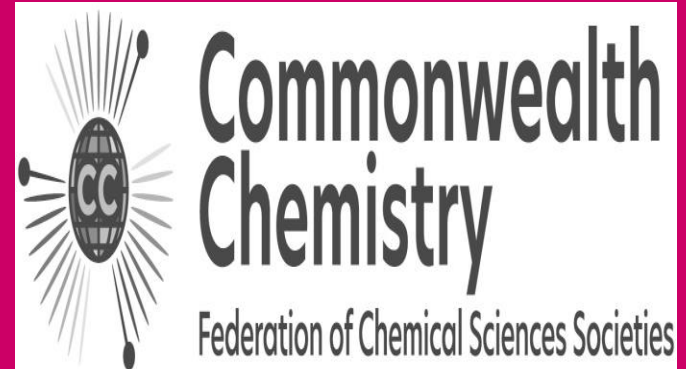


EVALUATION OF TRADITIONAL INDIAN FOOD GRAIN AS NEUTRACEUTICAL AND ITS ROLE IN AMLEORATION PCOS ASSOCIATED SYMPTOMS



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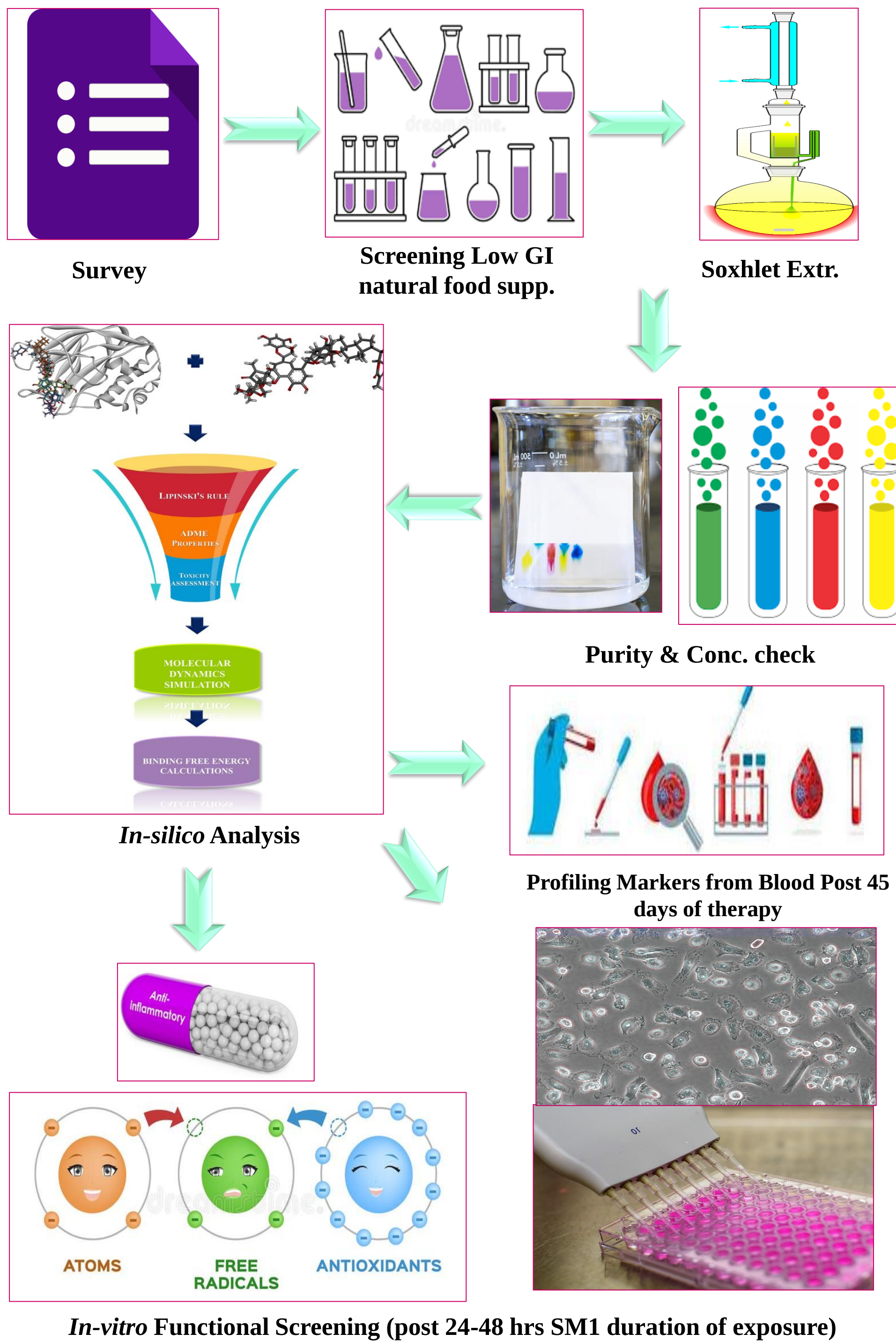
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ABSTRACT

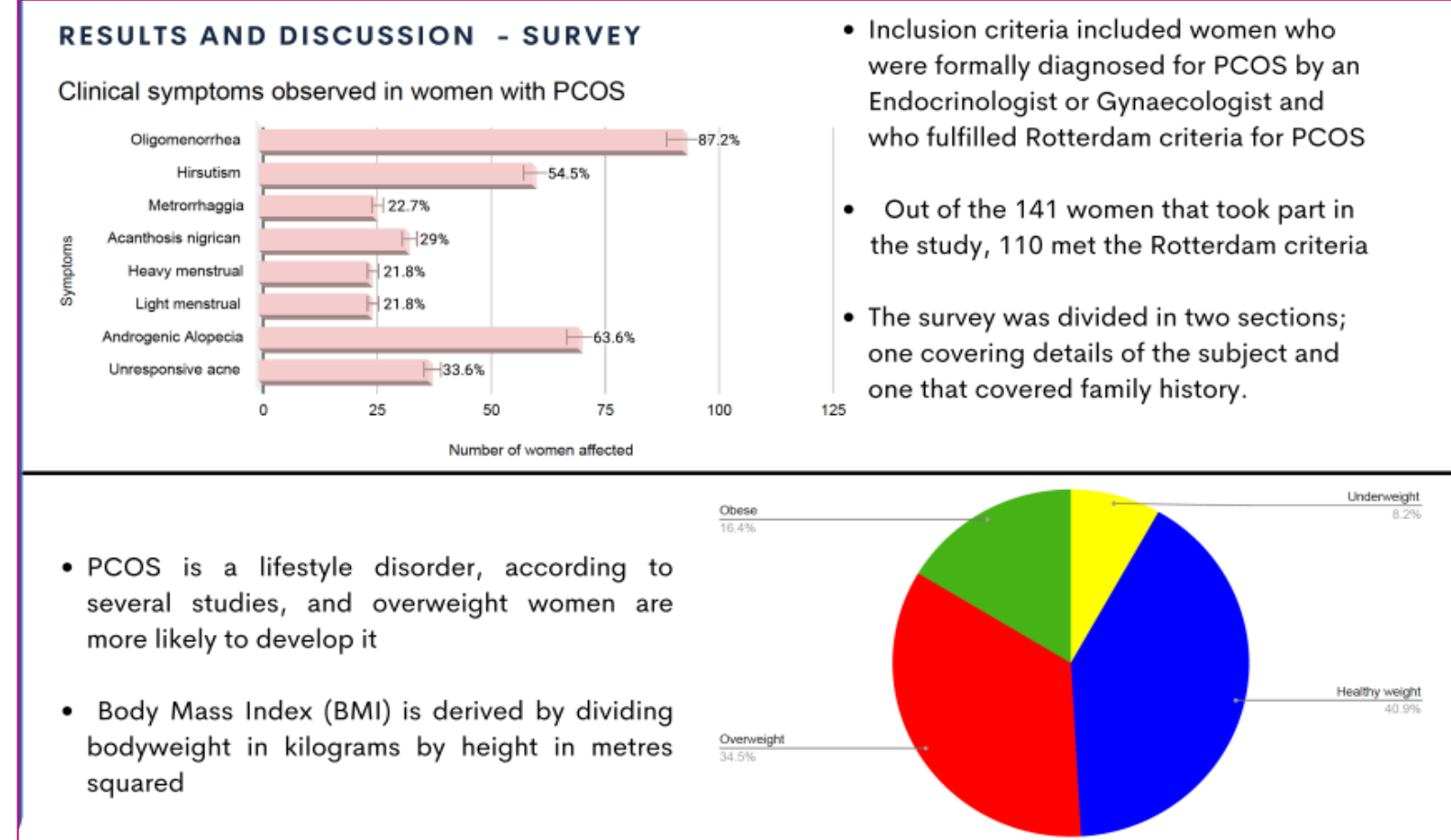
According to WHO; PCOS affects 3.4 percent of women globally, with 3.7-22.5 percent of women in India. PCOS has become one of most common cause of infertility, and while there are fewer medicines available to treat PCOS, those that do exist have undesired side effects. Contemporary research is focusing on natural supplements aka nutraceutical metabolites for ameliorating the PCOS associated inflammation, pain and infertility^[1]. The present study investigated the use of a traditional Indian dietary plant-based compound named ‘SM1’ to treat PCOS and its symptoms like inflammation, hyperandrogenism, insulin resistance, and dyslipidemia, among others. Commonly used plant-based flour samples were analyzed for their nutritional qualities and GI index, which indicated SM1 to be an optimum plant-based dietary product. The nitric oxide scavenging assay, DPPH free radical scavenging activity, H₂O₂ radical scavenging activity, and reducing power assay results all showed a reduction in oxidative stress which contribute to the pathophysiology of PCOS. The safety of product was evaluated employing *in-vitro* cytotoxicity assay on blood cell culture. IC₅₀ values obtained exhibited safety of product being analyzed. SM1 was also gauged to be a promising anti-cancer molecule with IC₅₀ 110.5 µg/ml for MCF-7 cell lines. Intermolecular hydrogen bond interactions between the chosen protein receptor, TNF-α and the ligand methyl ferulate were analyzed employing in-silico molecular docking and toxicity analysis^[2-4]. The nitric oxide scavenging assay, DPPH free radical scavenging activity, H₂O₂ radical scavenging activity, and reducing power assay results all showed a reduction in oxidative stress which contribute to the pathophysiology of PCOS. The total phenolic content for ethanolic extracts of SM1 compound was estimated to be 4.5mg/ml. The Nitric oxide radical scavenging activity for 50µg/ml concentration of SM1 extract was estimated to be 46.7% as compared to ascorbic acid which was estimated to be 29.3% for 50µg/ml concentration. The inhibition concentration of DPPH radical scavenging activity was found to be 54.4% as compared to inhibition concentration of ascorbic acid which was found to be 41.2% indicating anti-oxidative potential of SM1 extract. The SO radical scavenging activity of SM1 extract was found to be 47.8% as compared to inhibition concentration of ascorbic acid which was found to be 70.3%. The inhibition concentration of H₂O₂ radical scavenging assay for SM1 extract as seen in table 5 was found to be 66.8% as 46 compared to inhibition concentration of ascorbic acid which was found to be 50.3%. tested on 33 women detected with PCOS syndrome. Total testosterone, luteinizing hormone, and glycated haemoglobinA1c and anthropometry parameters all showed considerable changes, but lipid profile and follicle stimulating hormone only revealed minor variations.

Keywords: PCOS, inflammation, hyperandrogenism, insulin resistance, and dyslipidemia, Glycemic Index, TNF- alpha.

METHODOLOGY

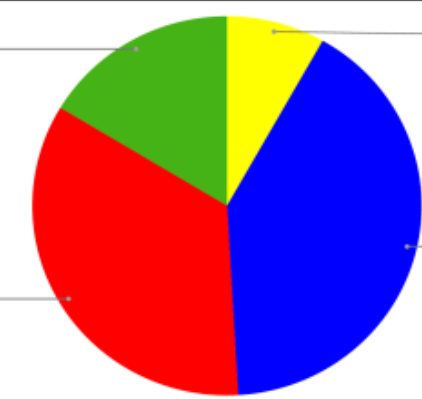


RESULTS & DISCUSSION

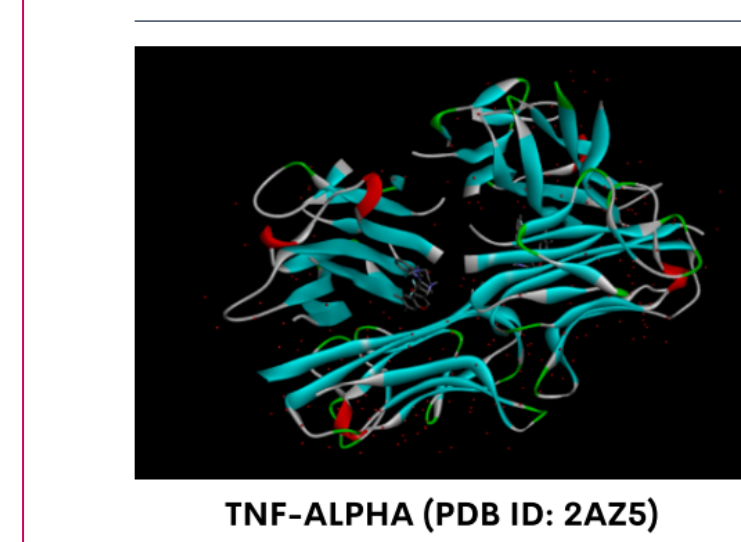


- PCOS is a lifestyle disorder, according to several studies, and overweight women are more likely to develop it
- Body Mass Index (BMI) is derived by dividing bodyweight in kilograms by height in metres squared

- Inclusion criteria included women who were formally diagnosed for PCOS by an Endocrinologist or Gynaecologist and who fulfilled Rotterdam criteria for PCOS
- Out of the 141 women that took part in the study, 110 met the Rotterdam criteria
- The survey was divided in two sections; one covering details of the subject and one that covered family history.



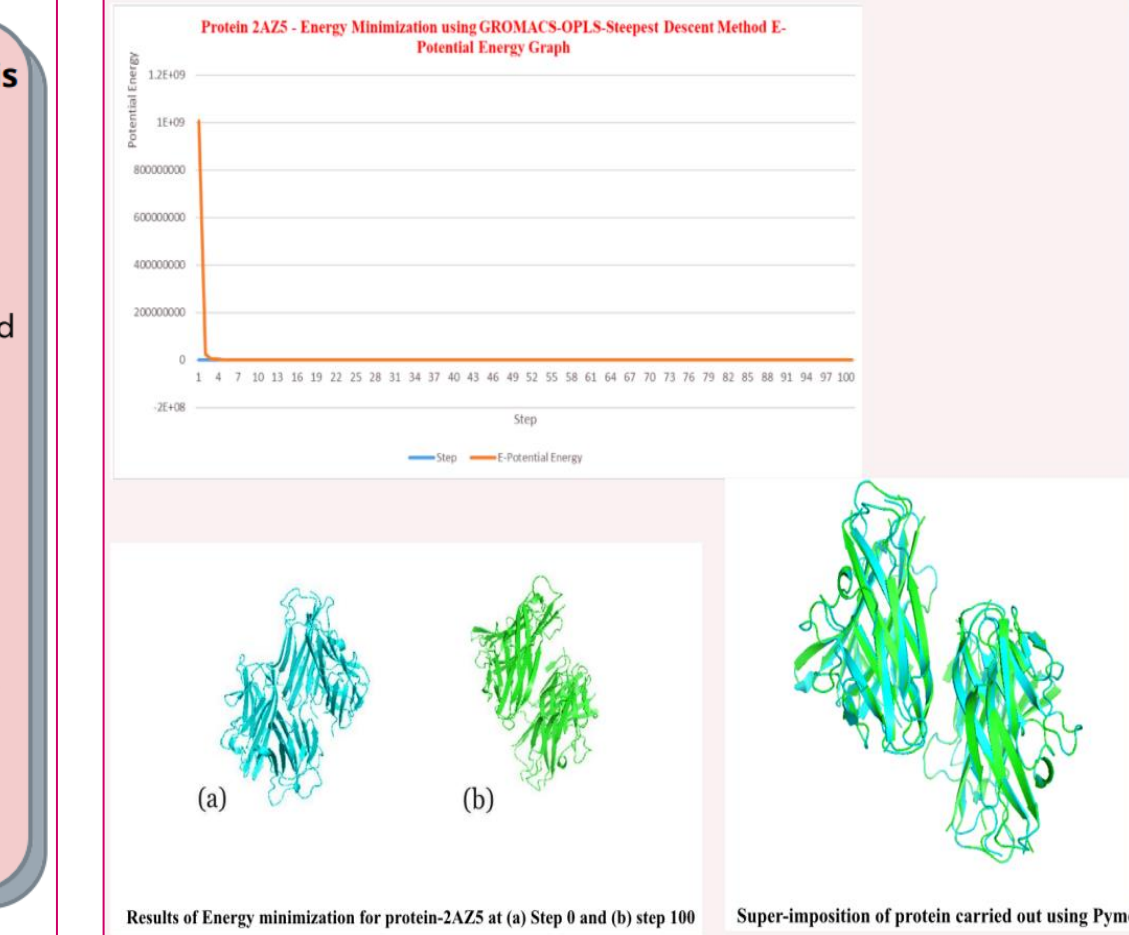
The participants were	
Included	Excluded
Pre-menopausal women with age between 17 and 40	If they were pregnant/ lactating mothers
Diagnosed formally by a medical professional	Post-menopausal women
Matched 2/3 criteria in Rotterdam score	Women with hysterectomy
	Patients with congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, androgen secreting tumours, hypogonadism and hypo gonadotrophic



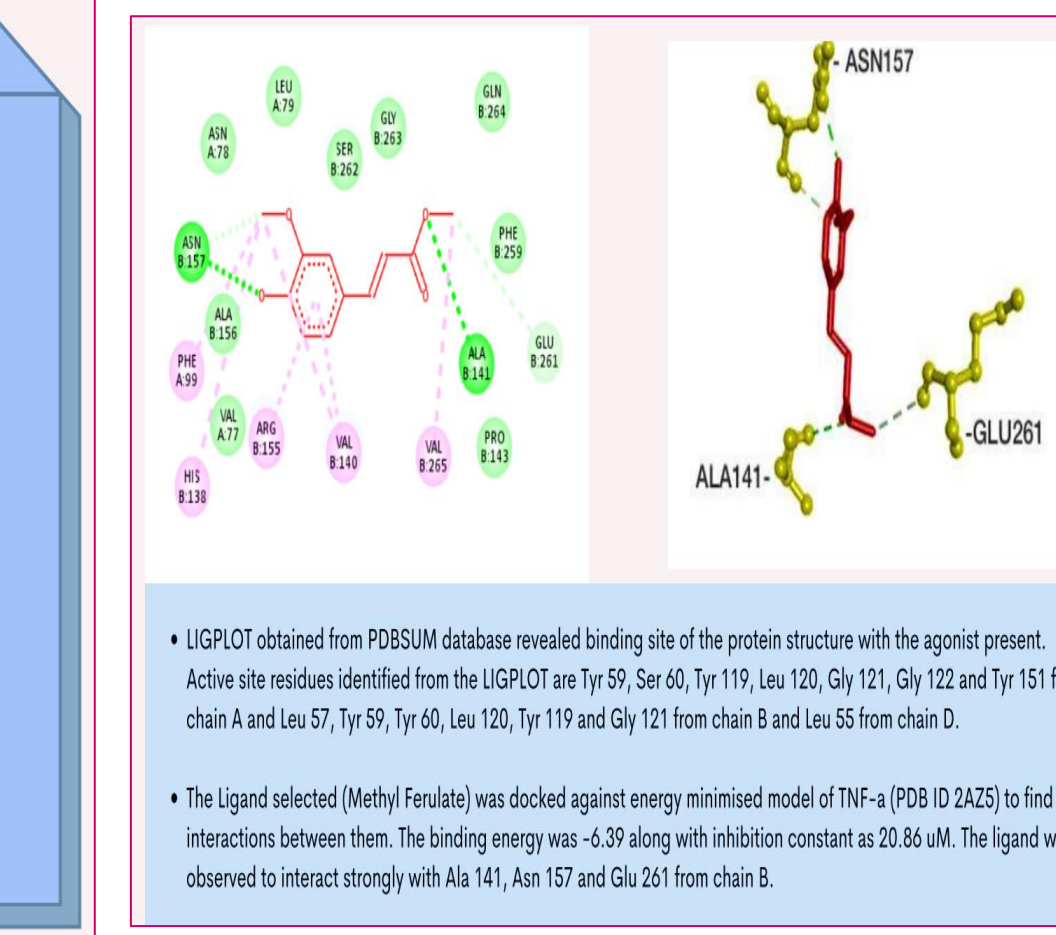
- Out of all structures on the Protein Data Bank, PDB ID 2A25 was selected on the basis of resolution.
- PDB entry is named as 'Crystal Structure of TNF-α with a small molecule inhibitor'.
- Crystal structure was determined by X-Ray diffraction method and has a resolution of 2.10 Å.
- Protein sequence for the protein was retrieved from the corresponding UniProt entry P01375 in FASTA format.

Formula	C ₁₁ H ₁₄ O ₄
Molecular Weight	208.21
#Heavy atoms	15
#Aromatic heavy atoms	6
#Rotatable bonds	4
#H-bond acceptors	4
#H-bond donors	1
ESOL Class	Soluble
GI absorption	High
BBB permeant	Yes
PCP substrate	No
CYP1A2 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
log Kp (cm/s)	-6.26
Lipinski violations	0
Bioavailability Score	0.55
Synthetic Accessibility	2.13

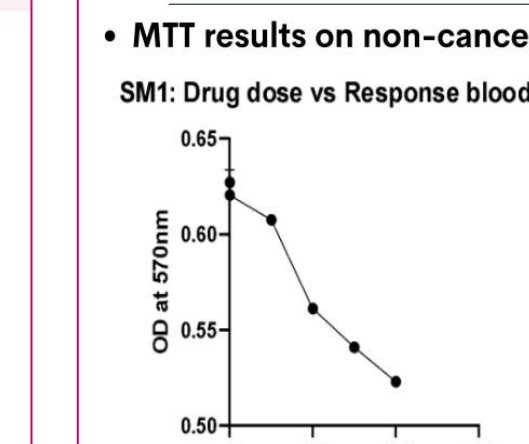
- **In-silico ADMET analysis**
- Methyl Ferulate was chosen as the ligand. It: has high GI absorption is permeable into blood brain barrier (BBB)
- shows no PGB substrate hence no efflux.
- has high drug likeness as Lipinski violation is zero
- is not an inhibitor to CYP class protein, metabolizing easily and can excrete easily.



- Energy minimization of protein 2A25 was performed to increase its stability and to obtain an energetically favourable state.
- The energy minimized model was analyzed to observe the trend of potential energy.
- The initial energy of protein at step 0 was found to be 1.00680e+09 and the final energy after minimization at step 100 was 6.357957e+04.
- Super-imposition of protein was carried out using PyMol and the RMSD value obtained was 0.063A which was found to be significant (< 1A).

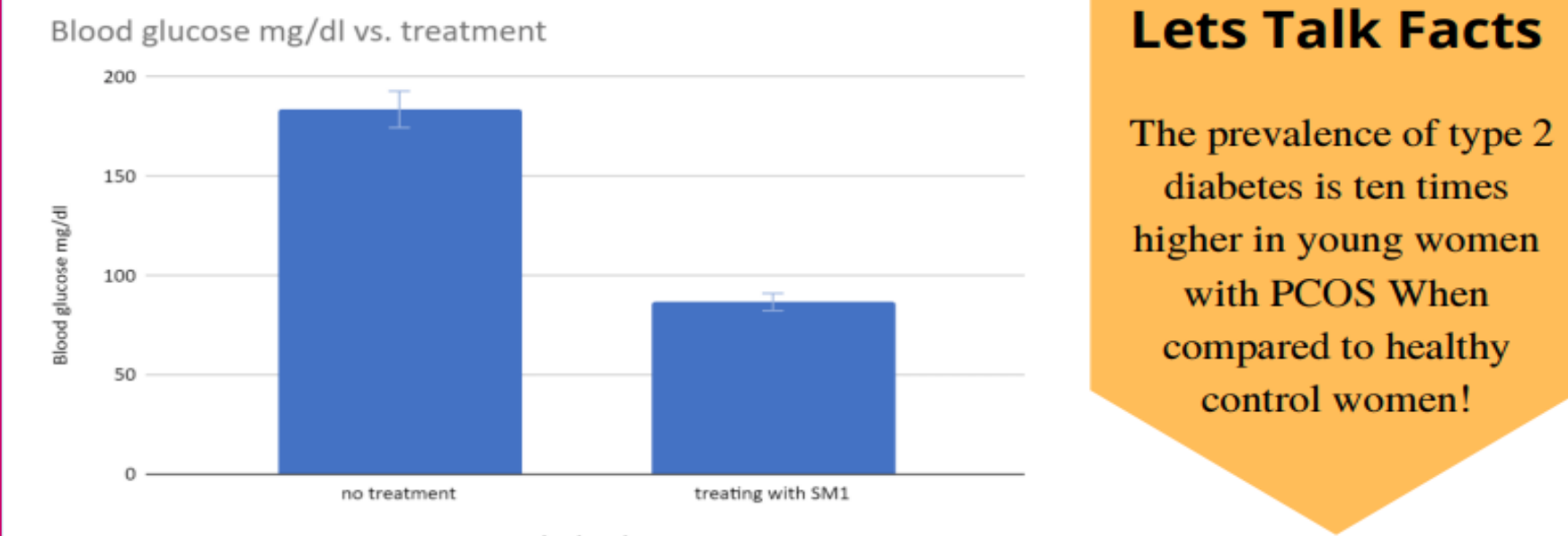


- UGT1A1 obtained from PDBTM database revealed binding site of the protein structure with the agonist present.
- Active site residues identified from UGT1A1 are: Asp 98, Ser 60, Tyr 100, Gly 102 and Tyr 101 from chain A and Leu 57, Tyr 59, Tyr 60, Leu 100, Tyr 119 and Gly 121 from chain B and Leu 55 from chain D.
- The Ligand selected (Methyl Ferulate) was docked against energy minimized model of TNF-α (PDB ID 2A25) to find interactions between them. The binding energy was -4.39 along with inhibition constant as 20.86 uM. The ligand was observed to interact strongly with Ala 141, Asn 157 and Glu 261 from chain B.



- **MTT results on non-cancerous blood cells:**
- SM1: Drug dose vs Response bloodcells
- A positive linear relationship can be observed between the concentrations of drug vs the inhibition of cells, as the concentration increases, inhibition of the cells also increases.
- A decreasing optical density indicates occurrence of cell death. The statistical analysis was obtained through Graph Pad Prism. Statistics of SM1 extract on blood cell line showed 95% profile likelihood. IC50 was calculated to be 167.0 to <infinity. The R-square value was calculated to determine the goodness of fit of the regression model.
- The value is expected to be in the range of 0 to 1 where, a value closer to 1 indicates the perfect fit on the regression line. The R2 value was observed to be 0.9736

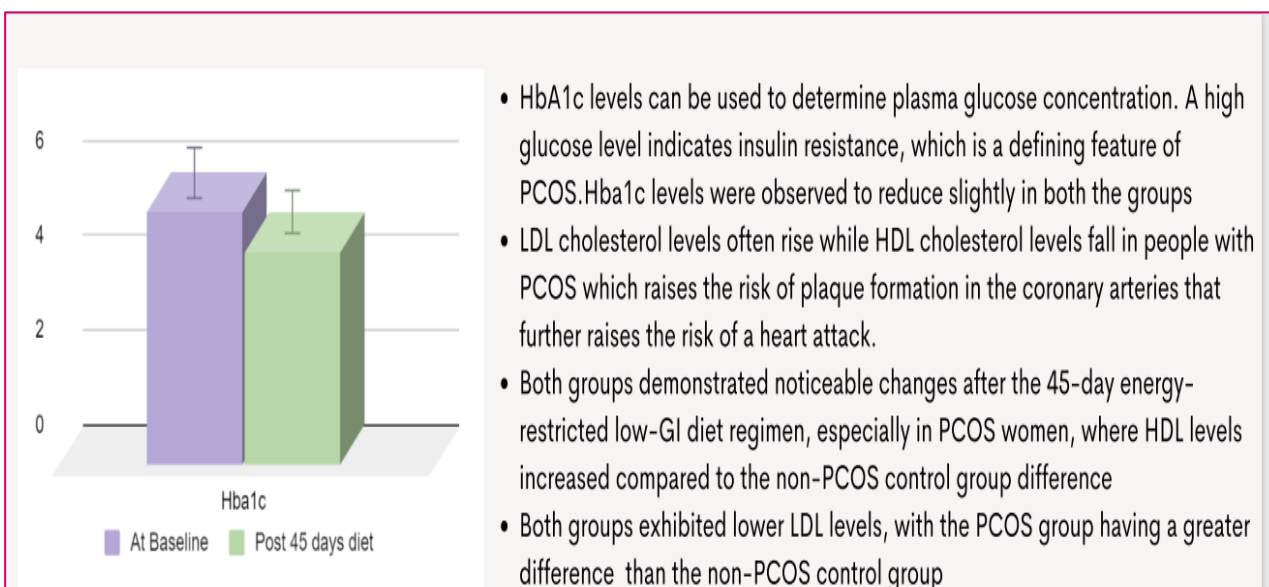
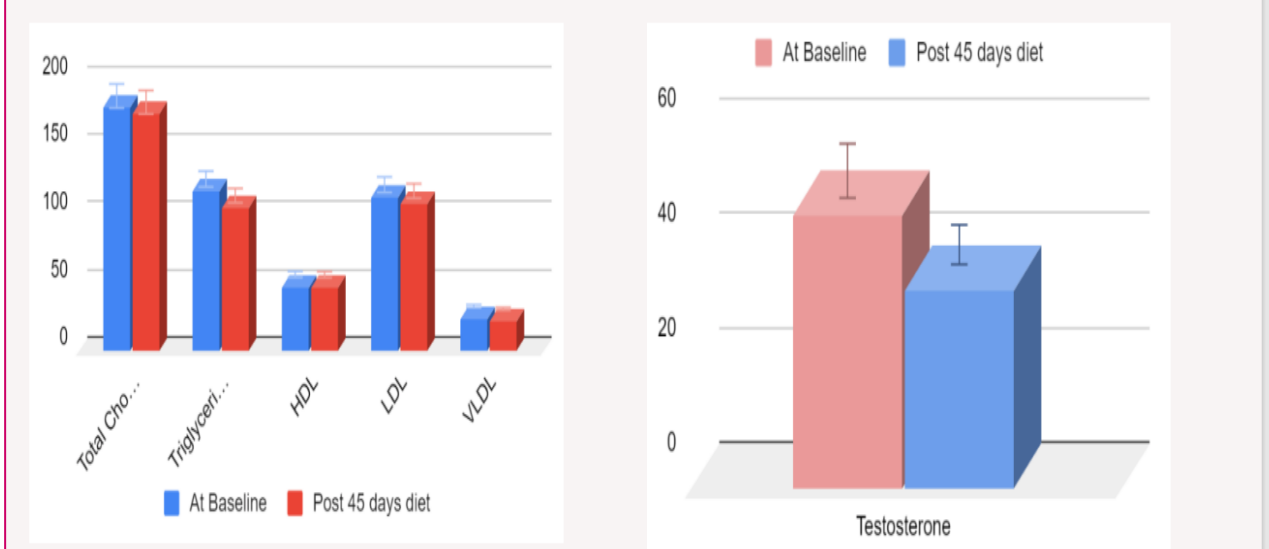
RESULTS AND DISCUSSION - GLUCOSE REGULATORY EFFECT



- Blood glucose levels of a diabetic patient dropped from 183.70 mg/dl to 86.72 mg/dl in under four hours testing with SM1 extract.
- Impaired glucose tolerance, obesity, type 2 diabetes, and an increased risk of cardiovascular disease are metabolic implications of PCOS.
- Insulin resistance, a unique characteristic of PCOS that affects 50–70% of women with the condition, is indicated by high blood glucose levels. Compensatory hyperinsulinemia is thought to produce hyperandrogenism as well as persistent oligo- or anovulation.

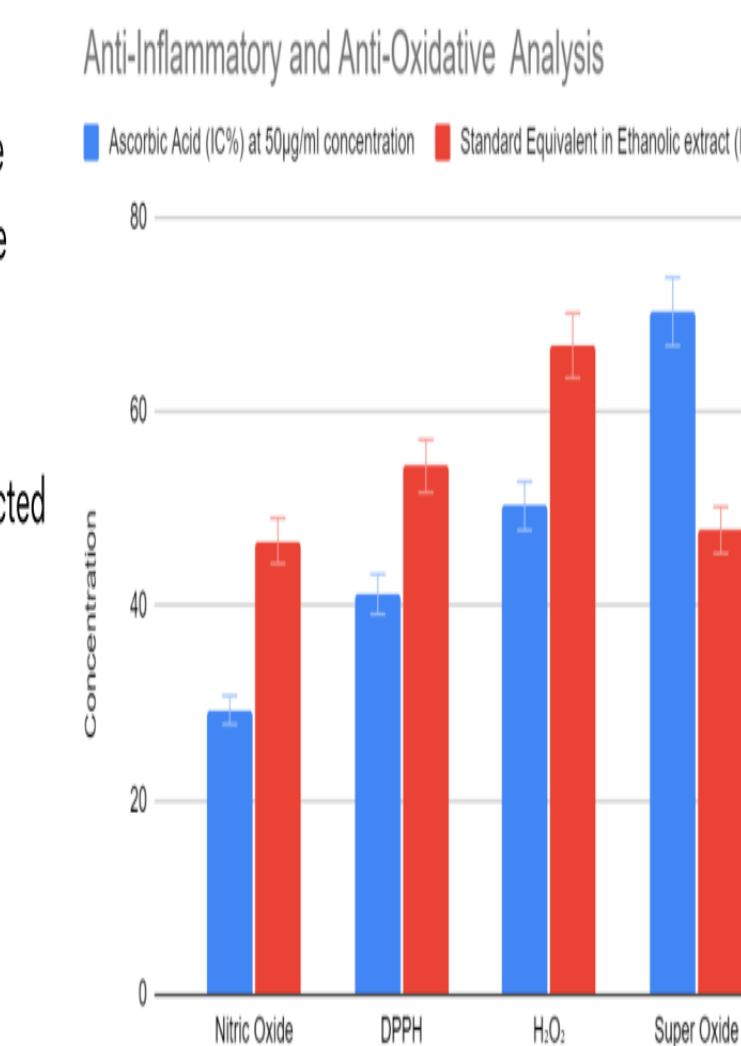
Conclusion

- The study endeavors to identify a natural food compound that ameliorates the symptoms associated with PCOS.
- TNF- α was also chosen as a receptor to be tested for the efficacy of the chosen ligand, Methyl ferulate, which demonstrated strong intermolecular hydrogen bonding at Ala 141, Asn 157, and Glu 261.
- On blood cells, the IC50 value was 167 µg/ml,
- The results of the nitric oxide scavenging assay, DPPH free radical scavenging activity, H2O2 radical scavenging activity, and reducing power assay showed a decrease in oxidative stress and reactive oxygen species (ROS), which are linked to insulin resistance and contribute to the pathophysiology of PCOS
- An energy-restricted low-GI diet plan was created, combined with a physical activity.
- The blood glucose values plunged from 183.70 mg/dl to 84.72 mg/dl
- The CRP values plunged from 23.09 mg/dl to 9.94 mg/dl post incubation for 24h at 37 degrees indicating anti-inflammatory effects of SM1.



Inhibitory concentration values for different tests performed

- Oxidative stress can be defined as an imbalance caused by excessive formation of oxidants in the presence of limited antioxidant defences.
- ROS and oxidative stress have been interconnected with insulin resistance
- Antioxidant treatments may improve insulin sensitivity in patients with insulin resistance and type 2 diabetes



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