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The Effect Of Oil Palm Phenolics (opp) And Curcumin On Plasma Metabolomic Profile In Atherogenic Diet Induced Rat Model Of Alzheimer's Disease (ad)

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THE EFFECT OF OIL PALM PHENOLICS (OPP) AND CURCUMIN ON PLASMA METABOLOMIC PROFILE IN ATHEROGENIC DIET INDUCED RAT MODEL OF ALZHEIMER'S DISEASE (AD)

by

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DEDICATION

I would like to thank my parents and my brother for their constant support, without whom I would never have been able to do anything. I would also like to thank my friend Debomitra Dey for her constant support throughout the journey.

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LIST OF ABBREVIATIONS

1H NMR	Proton nuclear magnetic resonance spectroscopy
Aβ	Amyloid-β peptide
AD	Alzheimer's disease
APP	Amyloid precursor protein
BBB	Blood Brain Barrier
CSF	Cerebrospinal fluid
IL-6	Interleukin 6
MRI	Magnetic resonance imaging
OPLS	Orthogonal projections to latent structures regression
OPLS-DA	Orthogonal partial least squares discriminant analysis
OPP	Oil palm phenolics
PCA	Principle component analysis
PLS-DA	Partial least square discriminant analysis
NFT	Neurofibrillary tangles

CHAPTER 1: INTRODUCTION

1.1 Alzheimer's Disease(AD)

Alzheimer's disease is a progressive neurodegenerative disease that includes symptoms such as memory loss, language deterioration which were discovered by Alois Alzheimer and his contemporaries. AD is one of the sixth leading cause for death in United states and fifth leading cause of death in Americans who are 65 years or older. The aggregation of B-amyloid plaques in the brain is the main characteristic of AD. Neuropathologically, AD is characterized by the presence of senile plaques, neurofibrillary tangles, persistent neuronal loss, although, the neurotoxic mechanisms have not been completely elucidated. Both oxidative stress and inflammation play a key role in the illness[1]. AD is often preceded by three stages of progression characterized by gradual increase of neuropathological hallmarks (SP and NFT) starting from preclinical AD (PCAD) to amnestic mild cognitive impairment (MCI) and early AD (EAD)[2]. Several hypotheses have been put forward on the basis of the various causative factors in order to explain this multifactorial disorder[3] such as the cholinergic hypothesis, Aβ hypothesis, tau hypothesis and inflammation hypothesis[4]. Currently there is no specific treatment for Alzheimer's Disease. Identifying quantifiable markers of disease—whether by neuroimaging, CSF, or blood markers—has the potential (a) to allow for early identification of possibly presymptomatic subjects at risk for developing AD and (b) to provide measures by which disease severity and progression may be evaluated; thus, such biomarkers may be invaluable in providing endpoints (or objective milestones) for clinical therapeutic trials[5]. There are different types of fluid biomarkers such as CSF-based biomarker and blood plasma based biomarkers which are used in the investigation of AD. A study by Ray S., et al., in 2007 proposed plasma biomarkers as an alternative to CSF biomarkers for the early detection of AD. The naturally occurring anti-inflammatory and anti-oxidant compounds including curcumin are currently under investigation. There are some diet related disorders such as hypercholesterolemia, diabetes, etc., which are also correlated to AD[6]. Many studies have recommended a positive connection between AD and the consumption of diet low in antioxidants[7].

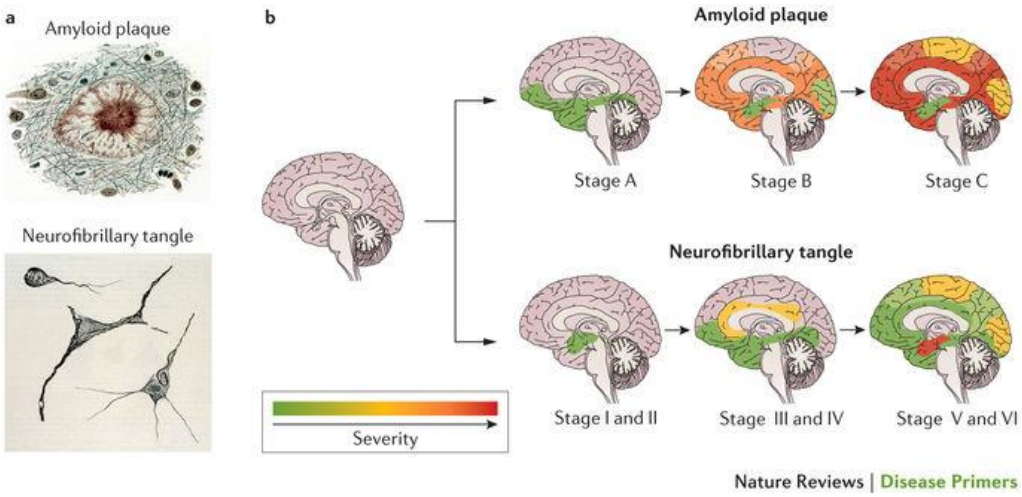


Fig 1: Amyloid plaques and neurofibrillary tangles spread through the brain as the disease progresses[6].

1.2 Oxidative stress

Numerous studies demonstrate that different biomarkers of oxidative-stress-mediated events are elevated in the AD brain. Studies in animal models of the disease with antioxidants report significant improvements of their AD-like phenotype [9]. Under normal conditions, damage by oxygen radicals is kept in check by an efficient array of antioxidant systems that display extensive redundancy (e.g. the simultaneous metabolism of H2O2 by catalase and glutathione peroxidase). However, during pathological conditions, the oxidant versus antioxidant balance is necessarily altered, either primarily or secondarily. Oxidative damage occurs when the oxidative balance is disturbed such that reactive oxygen production exceeds cellular anti-oxidant defenses. The oxidative damage found in AD includes advanced glycation end products [1,4], nitration [5,6], lipid peroxidation adduction products [7,8], carbonyl-modified neurofilament protein and free carbonyls [9,11]. Oxidative stress has been associated with the onset and progression of mild cognitive impairment (MCI) and Alzheimer disease (AD). AD and MCI brain and plasma display extensive oxidative stress as indexed by protein oxidation, lipid peroxidation, free radical formation, DNA oxidation, and decreased antioxidants.[10]

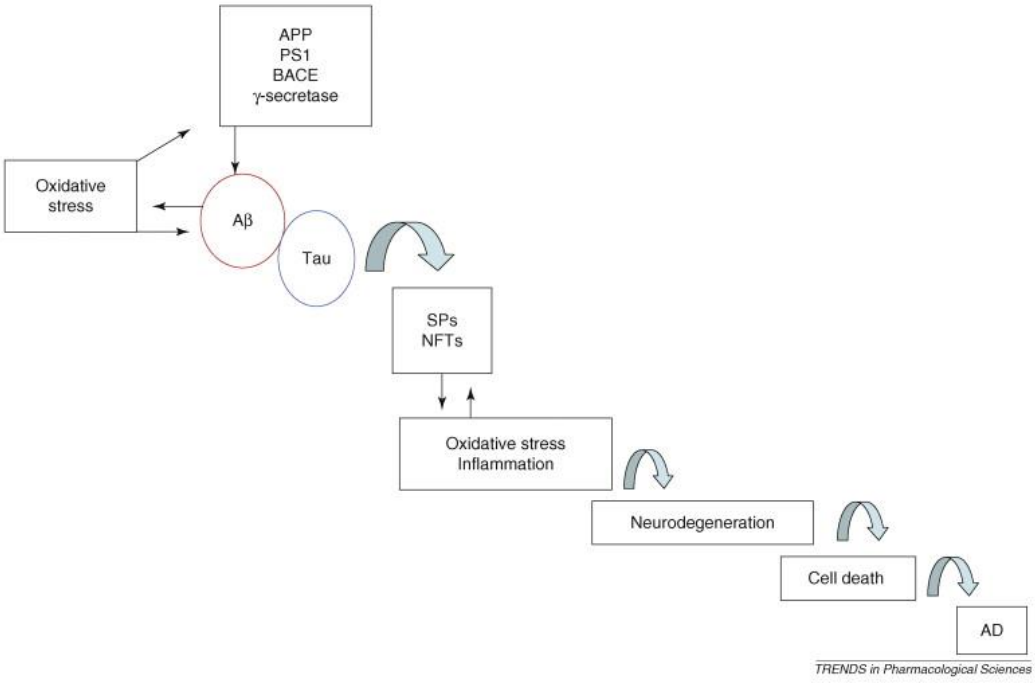


Fig 2: Oxidative stress in Alzheimer's Disease[9]

1.3 Oil Palm Phenolics (OPP)

Diets containing high amounts of phytochemicals can give security against free radical-induced diseases, because of their high cell reinforcement activities. Oil palm phenolics (OPP) is a complex fluid derived from plant which for the most part includes polyphenol mixes, shikimic acid, oligosaccharides, and lipid. Likewise, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid and ferulic acid are additionally nearness in OPP through superior fluid chromatography (HPLC), fluid chromatography-pair mass spectrometry (LS/MS/MS) investigations. Although the fruits of oil palm are mainly used for extraction of eatable oils, successful recuperation from the aqueous by-products following palm oil generation has recognized a water soluble complex rich in phenolics and natural acids altogether alluded as Oil Palm Phenolics. Plant phenolics are imperative antioxidants because of their high redox potential, which allows them to go about as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators. Antioxidants that gather in neuronal tissues are potential candidates for the anticipation and treatment of neuronal disorders involving oxidative

stress. Phenolic antioxidants might possibly cross the brain barrier, depending on their properties, such as charged state, lipophilicity, and interactions with efflux transporters, with possible relative specificity of the compounds for various brain areas. Numerous current studies have shown that plant phenolics have neuroprotective effects, through their capacity to decrease oxidative stress. Notwithstanding their cell reinforcement activities, plant phenolic compounds such as flavonoids have been shown to possess other atomic mechanisms [11]. Since AD and the associated amyloid plaque development has an underlying part of oxidative stress, we hypothesized that OPP may decrease amyloid beta weight in vivo. In a previous pilot study we discovered OPP would decrease be able to the β amyloid in the cell culture, it is necessary for us to start a further investigation on its impact by using a creature demonstrate with AD.

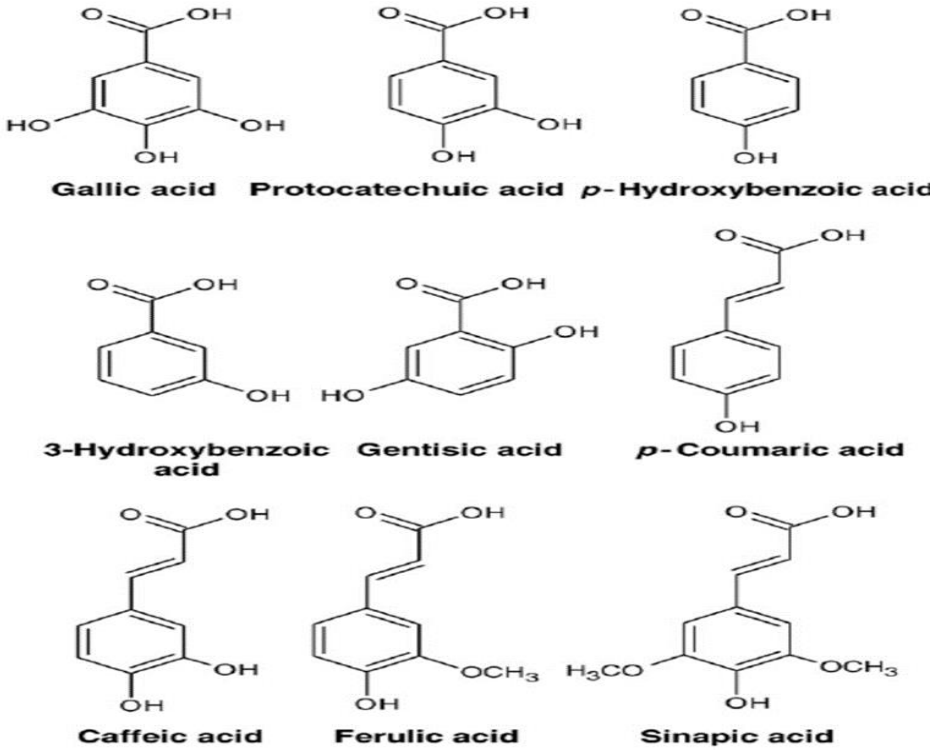
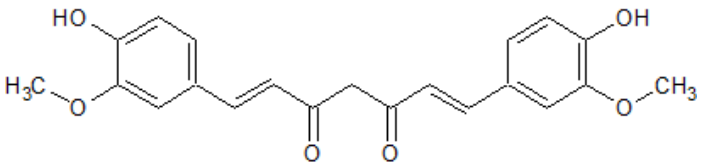


Fig 3: Main compounds of Oil Palm Phenolics[12]

1.4 Curcumin

Curcumin is extracted from the herb Curcuma Longa, more generally known as turmeric, a sterile plant. Formally recognized as diferuloylmethane, curcumin was first characterized in 1815, and its

crystalline shape was first acquired in 1870. For quite a long time, it was as often as possible utilized as a characteristic plant item in Ayurveda, an Indian arrangement of all-encompassing pharmaceutical, to treat inflammation, liver disorders, diabetic wounds, and Sinusitis. Its utilization has now extended past restorative purposes to numerous items and substances over the world, including curry, mustard, band-helps, cheddar, and spread[13]. Curcumin is a substance that is delicate to debasement by visible and ultraviolet light, and additionally high pH and oxygen, having a half-existence of around 8 hours in human blood[14]. The structure of curcumin is made from a carbon chain connecting two aryl gatherings. Scientists have discovered the phenolic OH bunches connected to the aryl gatherings to have the capacity to scavenge reactive oxygen species (ROS), adding to its hostile to oxidative impact. Moreover, curcumin was appeared to associate with various substances including DNA, lipids, and proteins[15]. The expanding measure of writing over Curcumin demonstrates adaptable uses of Curcumin's properties. Curcumin has been appeared to restrain various pathways required in apoptosis and cell invasion, exerts potent antioxidant effects, and influences catalysts that control tumor movement. In fact, explore has been done on the effects of curcumin for different therapeutic uses, and researchers have discovered potential parts of curcumin in restraint of invasion by thyroid disease cells, effort of defensive effects against alcohol instigated lethality, and aversion of bosom tumor, among different employments[16]. Curcumin's capacity to cross the blood-brain barrier (BBB) is basic to its improvement as a neuroprotective operator. In spite of the fact that it has been suggested that the capacity of dietary curcumin to manage the cost of neuroprotection in preclinical animal models of neurodegenerative diseases is proving its capacity to cross the BBB, this matter requires significant investigation[17].



Curcumin

Fig 4: Structure of Curcumin

1.4.1 Curcumin for Alzheimer's Disease:

Given the significance of β-amyloid amassing in the pathogenesis of AD, numerous in vitro and in vivo studies have examined the interaction of curcumin with β-amyloid. Several studies have investigated the dose-related neuroprotective impact of curcumin against β-amyloid-induced danger in refined neuronal cells[1, 17, 18]. Curcumin may also influence the creation and deposition of β-amyloid, long idea to be one of the triggers for neurodegeneration in AD. In both rodent cortical neurons and in solution, curcumin created a dose-subordinate decrease in arrangement of fibrillary β-amyloid1–40 and β-amyloid1–42 and furthermore destabilized fibrils that had effectively framed, thus breaking up the β-sheet adaptation seen in AD plaques[18]. Based on the various findings support the benefits of its oral intake, it is trusted that curcumin will prompt a promising treatment for Alzheimer's disease. In this way, we included curcumin in our trial elevated cholesterol eating regimen to serve as a positive control, which enables us to compare it and our testing supplement OPP in their helpful effects and potential mechanisms.

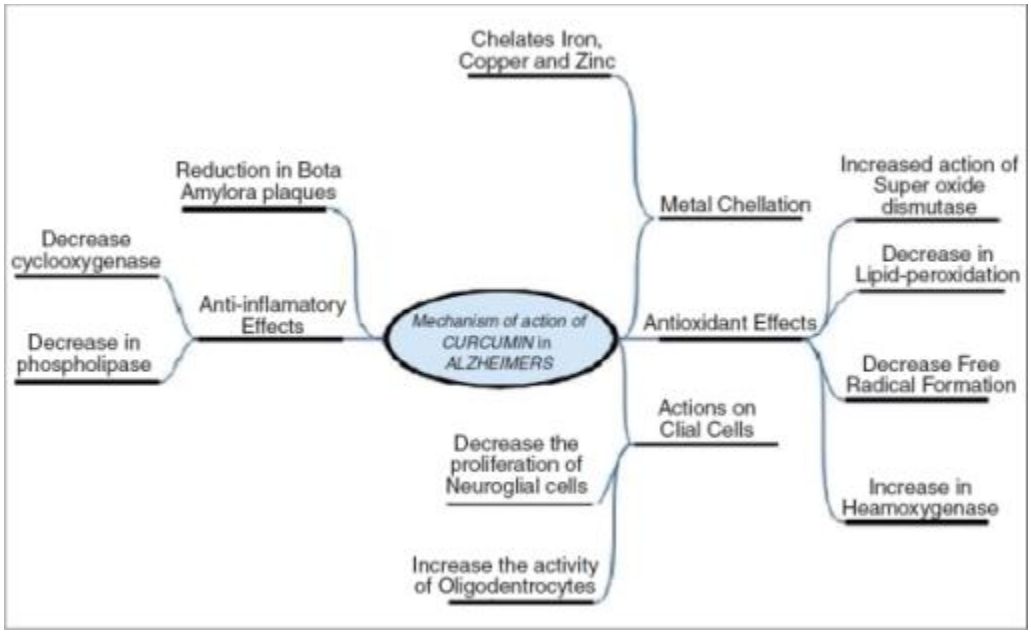


Fig 5: Different mechanisms of Curcumin in Alzheimer's Disease[19].

1.5 Metabolomics:

Metabolomics alludes to worldwide examination of small molecular weight molecules or metabolites in body fluids, for example, urine and plasma serum. Metabolites are intermediates or results from metabolism and are included in typical development, advancement and multiplication through different metabolic pathways. The human serum metabolome comprises of 4200 unique mixes while the human CSF contains almost 500 distinct metabolites. These metabolites can be lipids, amino acids, glucose or intermediates of digestion[20]. Metabolome is alluded to the gathering of metabolites at the season of testing. Distinguishing proof of the subsequent metabolic examples in biological fluids is viewed as metabolic profiling. Metabonomics ordinarily alludes to the estimation of changes in a life form's metabolic profile because of external stimuli, genetic modification or as an outcome of the nearness of illness. Metabolic profiling gives an exhaustive perspective of the physiology of that cell around then. Metabolites are included in all the cell exercises, for example, cell signaling, vitality exchange and correspondence. Metabolites are the downstream aftereffect of genetic translation forms and, all the while, reflect natural and way of life considers and in addition singular attributes identified with dietary reaction and gut microflora[21]. Researchers of the college of Alberta and the college of Calgary finished the primary draft of the human metabolome in January 2007; they have recorded around 2500 metabolites, around 1200 medications and 3500 sustenance segments which can be found in the human body. Metabolome is the gathering of all metabolites in the cell and human body contains trillions of cells so there may be the same number of little metabolomes, all can be not the same as each other.

1.6 Metabolomics and Metabonomics:

Metabonomics is the term given by Dr. Nicholson and his associates in 1996 to depict the metabolite profiling in biofluids. All in all, Metabonomics allude to the term used to depict an approach of concentrate all cell metabolome at one time. As per Dr. John Lindon Metabonomics term supplements genomics and proteomics.

Term metabolomics has more noteworthy similitude to metabolite and is more boundless at NIH and subsidiary researchers. As indicated by Nicholson this can be viewed as subset of Metabonomics and it covers ordering tests, understanding biochemical systems, distinguishing biomarkers, quantitatively breaking down fixations and fluxes and examining atomic flow and association[22]

1.7 Techniques and approaches for data acquisition:

As portrayed over, one of the goals of metabolomics in the pharmaceutical field is to recognize and evaluate particular metabolic markers defenseless to enhance quality control, enhance early conclusion, review restorative results and encourage the improvement of novel medications competitors[23]. The system depends on differential metabolic expression profiling. There are two general methodologies, target particular and worldwide or non-coordinated, for metabolomic biomarker disclosure. Target-particular or science driven, an approach habitually utilizes mass spectrometry and is utilized for approval and routine clinical examination. The major systems utilized for this approach incorporate Nuclear Magnetic Resonance (1H NMR) spectroscopy, Gas chromatography-mass spectrometry (GC-MS), Liquid chromatography-mass spectrometry (LC-MS). Barely any different procedures are additionally utilized for information obtaining including Fourier transformed infrared (FT-IR) spectrometry, high-performance liquid chromatography (HPLC) or capillary electrophoresis. Worldwide/non-directed methodologies may have more potential for biomarker disclosure since they are unprejudiced. They enable the concurrent assessment of hundreds to thousands metabolites in a visually impaired way without from the earlier expanding the potential number of hopeful biomarkers[24].

1.8 NMR Spectroscopy:

NMR Spectroscopy has been utilized for deciding the structures of natural compound for as far back as fifty years. It is a noninvasive and financially savvy method. Test measure required for NMR spectroscopy is generally bigger than those required for mass spectroscopy[25]. The foremost behind this scientific instrument utilizes attractive properties of a few cores. The atoms with an odd number of protons or neutrons have the attractive minute. Ordinarily, C-13 and H-1 are utilized for measuring reverberation

however other cores can likewise be utilized. Attractive cores, when subjected to attractive field particular to those cores, can ingest radio recurrence. Because of this absorbance, the cores are in reverberation state. These reverberation frequencies are distinctive for various particles and iotas inside atoms and this gives the auxiliary data about the particle. Two sorts of techniques are utilized ordinarily to get proton NMR spectra. One is called consistent wave strategy and the other is beat Fourier change system. In nonstop wave strategy, the example is put between the posts of an effective magnet and is spun while differing the attractive field quality. Proper radiofrequency is connected through the receiving wire curl and the reverberation of assimilated vitality is gotten by the beneficiary loop and recorded by an electronic gadget associated with the PC. Each synthetically unique proton in an example, when set in a solid attractive field will display a particular compound move which relies on upon the centralization of the proton in a particular domain. The distinctive metabolites will have protons in a synthetically unique condition and consequently will deliver the signs at various compound move esteems in the NMR range. Range can be dissected by doling out compound move to the metabolites and after that looking at them between the gatherings. In pulse Fourier change technique, the attractive field is kept consistent and the radio recurrence radiation of fluctuating recurrence is connected. The reverberation is recorded through the same route by beneficiary curl as in persistent wave technique. If there should be an occurrence of heartbeat Fourier change technique, a mind-boggling covering reverberation is gained in time-recurrence space known as free acceptance decay(FID) arrangement and after that these FID records are prepared to the recurrence area customary range by Fourier change. This range gives data on the number and kind of substance elements in a particle. Fourier change technique was used in this examination for assessment of contrasts in metabolomic profiles in atherogenic eat less carbs actuated rodent model of Alzheimer's (Joseph Hornak, www.cem.msu.edu/~reusch).

1.9 Metabolomics and Alzheimer’s Disease:

Metabolomics of biological fluids followed by multivariate analysis of the spectroscopic data is a systems biological approach that has been used to identify important changes in metabolism. NMR

metabolomics of transgenic AD mice model was used to identify and characterize small molecules that are changed in the urine levels during the AD development. Levels of 3-hydroxykynurenine, homogentisate and allantoin were significantly higher compared to control mice in the stage prior to AD symptoms and reverted to control values by early/middle stage of AD. The level of these changed metabolites at very early period may provide an indication of disease risk at asymptomatic stage[26].

Hypothesis:

The Oil Palm Phenolics (OPP) have shown anti oxidative and anti-inflammatory effects on Alzheimer's Disease. Wu Yan et al., demonstrated that dietary OPP has potential in enhancing an age-related decrease in spatial cognizance and Aß deposition in the hippocampus[27]. Therefore, we hypothesized, atherogenic diet (2% cholesterol) will elicit the AD neurotic changes in aging rats, and OPP will slow down the process by antioxidant & anti-inflammatory effects, which will be reflected in regulation of plasma biomarkers and pathways involved in AD.

We used Curcumin as a positive control and its impact on AD will be analyzed too in this examination. The accompanying specific aims were proposed to meet our theory

Specific Aim 1A: To investigate differences in plasma metabolomic profiles of Atherogenic Diet Induced Rat Model of Alzheimer's Disease (AD) and treatment with dietary OPP/ curcumin in Control (C), High cholesterol (H), High cholesterol + 5% OPP (HP) and High cholesterol + 2% curcumin (HC). To determine the correlations between the plasma metabolomic profiles and oxidative and inflammatory markers;

Specific Aim 1B: To identify and quantify metabolites responsible for the differences in Metabolomic Profiles of Control and treated groups.

Specific Aim 1C: To study the correlation of inflammatory markers and oxidative stress markers with the plasma metabolomic profile.

Specific Aim 2: To identify the pathways involved in the differences in the plasma metabolites markers.

CHAPTER 2: METHODOLOGY

2.1 Animals

24 in-bred Brown Norway (BN) rats were obtained from the aged rodent colonies of the National Institute of Aging (Bethesda, MD). Upon arrival, 12 rats were 22 weeks old and 12 were 24 weeks old.

2.2 Housing and Husbandry

All the rats were accommodated in individual cages, in the same room. They were housed at Laboratory Animal Resources (DLAR) facility under standard conditions. These conditions were approved by Wayne State University Animal Investigation Committee (AIC). All the animals received alternating light conditions with 12 hours of light and 12 hours of darkness, under normal humidity and at room temperature. Cage bedding and water were replaced weekly and their health were monitored regularly.

2.3 Experimental protocol and diets

Prior to the start of scrutiny, all the animals were allowed to reconcile for a week. Ensuing the reconciliation period, all the animals were randomly allotted in 4 different groups, with the constraint of same mean body weight for all the diets groups. The groups were named as per the diet: Control diet (n=8), High Cholesterol diet (n=8), High Cholesterol + Curcumin diet (n=8) and High Cholesterol + OPP (n=8). The diet compositions are summarized in table 1. Isocaloric diet was provided to all the groups. The purified and sufficient diets were obtained in pellets from Dyets Inc. (Bethlehem, PA), for the entire duration of the study. The diets were kept at -20°C and diet was taken weekly as needed and kept refrigerated at 4°C. Animals were fed ad libitum and had free access to water.

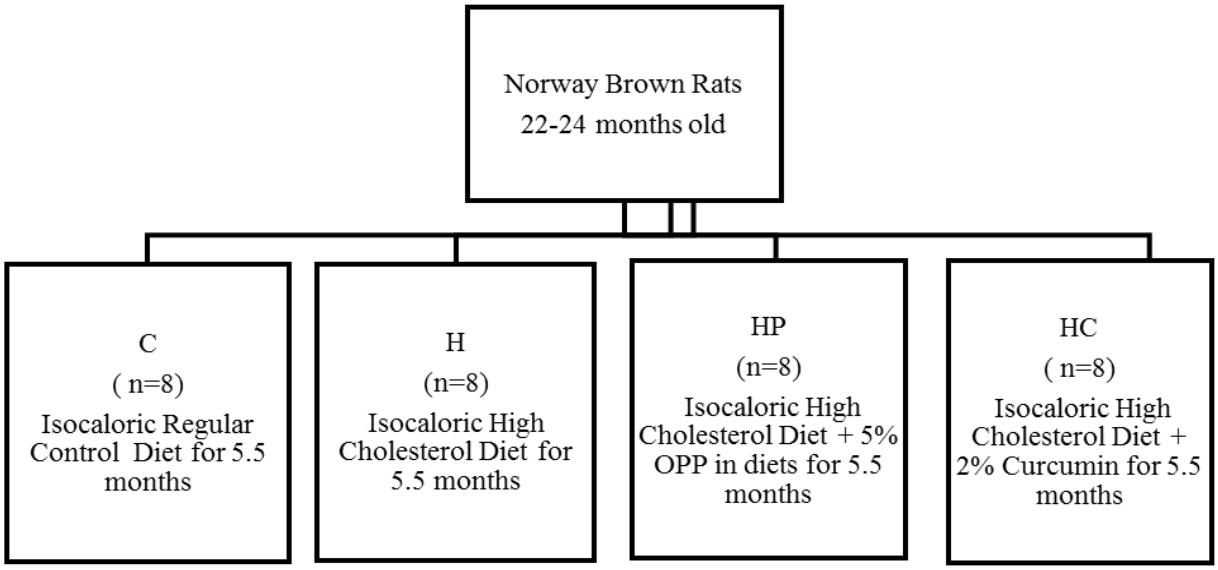


Fig 6: The different groups of the animals based on the atherogenic diet

Control groups

C: Rats on standard purified diet.

Experimental groups

H: Rats on standard purified diet + 2% cholesterol;

HP: Rats on standard purified diet + 5% OPP + 2% cholesterol;

HC: Rats on standard purified diet + 2% Curcumin + 2% cholesterol.

2.4 Experimental Procedures

The table 2 summarizes the experimental procedures carried out for the study. All the rats were provided with their respective diets for 23 weeks and had free access to water. Body weight and food intake were recorded twice a week throughout the duration of the study. Upon completion of the study at week 23, each animal was anesthetized using carbon dioxide chamber and decapitated followed by

exsanguination and tissue collection. Tissues were flash frozen in liquid nitrogen then stored at -80°C until ready to be used for analysis. Plasma was isolated and stored at -80ºC until ready to be used for analysis. All procedures and protocols were in accordance with and ratified by the Animal Investigation Committee of Wayne State University.

Table 2: Experimental procedures

Procedures	Frequency of Measurement
Body weight, diet intake, water intake	Twice weekly (week 1-6)
Urine collection	Once weekly (Week 2,4,6)
Plasma and tissue collection	End of study (Week 6)

Table 1: Composition of purified diets

Ingredient	Isocaloric Control	High Cho (2% Cholesterol)	High Cho +5%OPP	High Cho+2% Curcumin
		g/kg		
Casein	140	140	140	140
L-Cystine	1.8	1.8	1.8	1.8
Sucrose	100	77.5	77.5	77.5
Cornstarch	465.692	465.692	415.692	445.692
Dyetrose	155	155	155	155
Soyabean oil	40	40	40	40
t-butylhydroquinone	0.008	0.008	0.008	0.008
Cellulose	50	50	50	50
Mineral Mix#210050	35	35	35	35
Vitamin Mix#310025	10	10	10	10
Choline Bitartrate	2.5	2.5	2.5	2.5
Cholesterol	_	20	20	20
Cholic Acid	_	2.5	2.5	2.5
OPP	_	_	50	_
Curcumin	_	_	_	20
Total	1000	1000	1000	1000
Calorie	3602	3512	3332	3440

Diets were prepared and pelleted by Dyets Inc. (Bethlehem, PA).

Overall Study Design

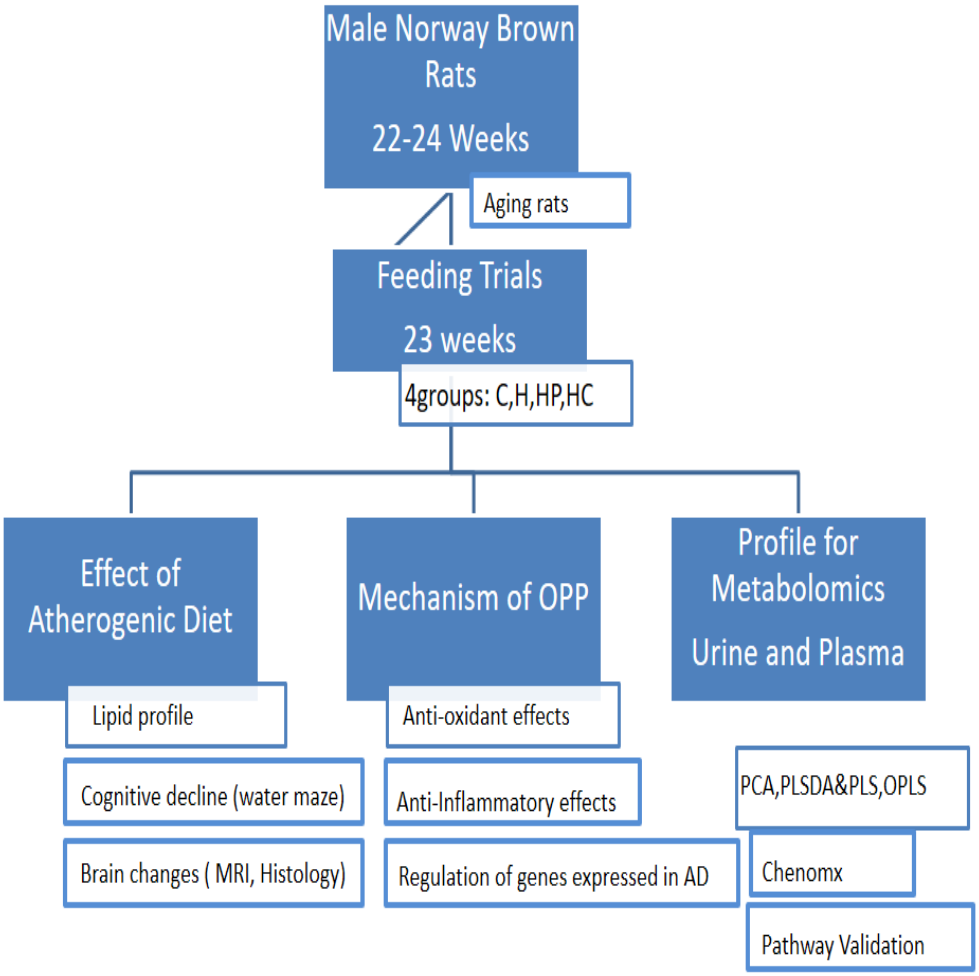


Fig 7: The overall study design

2.5 Sample preparation for NMR spectroscopy:

Plasma samples were thawed from -80°C and equilibrated to room temperature. On the bases of amount collected, samples were selected for NMR Spectroscopy. Total 20 samples were selected, and were divided into group of 5 based on the diet. The grouping is as follows: Control (n=5), High Cholesterol diet (n=5), High Cholesterol + Curcumin diet (n=5) and High Cholesterol + OPP (n=5).

2.6 NMR Spectroscopy:

Plasma samples from all the groups were analyzed on Varian 600S NMR Spectrometer (Varian Inc, Palo Alto, CA, USA) at 600 MHz. The probe used was 5mm AutoX Dual broadband with variable temperature capabilities. VNMRJ software was used. Console was multinuclear broadband system, pulse field gradient and variable temperature compatible. ACD software (ACD Toronto, Ontario, Canada) was used for proper processing of the spectra for subsequent multivariate analysis using SIMC P+ software (Umetrics, Kinnelon, NJ). All the free induction decay (FID) files from NMR spectrometer were imported at one time to the ACD software to avoid differences caused by spectral processing at different times. The fid files were fourier transformed, baseline corrected and auto phased. Spectra were calibrated using DSS peak as reference at 0 ppm (parts per million). Then Spectra were binned into group 1000 bins using intelligent binning. We used intelligent binning to make sure that a single peak would not be split into different bins. The table of integrals was exported to SIMCA P+ software for principal component analysis (PCA). Binning divides the spectra into small regions and the table of integrals can be analyzed by multivariate analysis as different variables.

2.7 Multivariate analysis:

Using SIMCA-P+ software, both multivariate pattern recognition techniques, unsupervised (principal component analysis, PCA) and supervised (partial least-squares discriminant analysis, PLS-DA) were employed to the data in order to discriminate sample spectra of different experimental groups. PCA is an unsupervised mathematical algorithm. A principal component (PC) is weighted linear combination of each of the original NMR variables so that the original data matrix is compressed into a smaller number of

variables; normally NMR data is compressed into 3-4 PC's. The weight given to each variable within a PC describes how influential that variable is in relation to the other variables. We used PCA to examine inherent clustering and correlations within the data. Spectral region 4.5-6 ppm from table of integrals was removed prior to PCA analysis as this region contains water peaks and other aliphatic compound with exchangeable protons. Pareto scaling was used to see the small changes in metabolite concentration between the two groups. PCA is an unsupervised multivariate projection method designed to extract and display the systemic variation in the data matrix X as a score plot. The corresponding loading plot provides information about the part of the spectrum that is responsible for the similarities and/or dissimilarities in the data set as observed in the score plot. PLS is a regression extension of PCA, which was used to connect the information in two blocks of variables, X and Y, to each other. Moreover, regression analysis using orthogonal projections to latent structures (OPLS) was also conducted on several investigated variables. Regression analysis enables the evaluation of the relationship between urinary metabolite profiles with the variables investigated independently of the metabolomic profiles. In PLS-DA the data set was distributed into classes and its objective was to find a model that separated the classes of observation on the basis of their X-variables, while using a hypothetical Y-variable. Both OPLS and PLS-DA methods of analysis are supervised, which implies that some information about the data set is provided to the software prior to analysis.

2.8 Metabolite identification and quantification:

The fid files from Varian 600S NMR spectrometer were imported to the CHENOMX suite (CHENOMX INC, Edmonton, Alberta) for the measurement of the metabolite concentration. Imidazole added in the preparation of samples was used as pH indicator and DSS as reference at 0 ppm. The spectra were Fourier transformed and processed using the processor tool within the software. This included base line correction; auto phasing and reference deconvolution. These processed files were analyzed in profiler module of the software. The CHENOMX Profiler module uses targeted profiling where spectral binning or spectral bucketing is not required. Targeted profiling is unique as it has the ability to analyze one compound

selectively or selective peaks individually for the spectrum (Colin Vitols, Ryan Rosewell, Identifying metabolites in biofluids, March 2006, CHENOMX publications).

Clusters can be selected for analysis (CHENOMX Publication). We used DSS as chemical shape indicator. Peaks and clusters from the spectrum were fitted using 500 library which is essentially NMR database of more than 250 metabolites. Concentrations of the metabolites were measured by fitting the peaks in the sample spectrum to a reference spectrum by click and drag kind of interface. The compound selected appeared as series of multiple dots on the spectrum, peak is fitted by dragging the arrows sideways. In the area crowded with clusters, multiple compounds needed to be fitted to match the spectrum as closely as possible. Calculation of the concentrations was based on the concentration of DSS, the chemical shape indicator. Metabolic pathways for the metabolites identified were searched through KEGG ligand database.

2.9 Pathway Validation:

MetaboAnalyst3.0 software was used to explore the potential pathway. MetaboAnalyst software utilizes pathway enrichment analysis and pathway topology analysis to translate metabolic trends into defined pathways relevant to the study. MetaboAnalyst likewise bolsters various data analysis and data representation errands utilizing a scope of univariate, multivariate techniques, for example, PCA (principal component analysis), PLS-DA (partial least squares discriminant analysis), warm guide bunching and machine learning strategies. MetaboAnalyst likewise offers an assortment of apparatuses for metabolomic data translation including MSEA (metabolite set enrichment analysis), MetPA (metabolite pathway analysis), and biomarker selection via ROC (receiver operating characteristic) curve analysis, and in addition time arrangement and influence analysis. A .csv or .txt document is typically utilized when the entire data set has already been pre-prepared to a tabular frame, for example, a compound concentration table, a spectral bin table, or a peak force table. The compress record format is typically used to upload numerous MS spectra or various peak list documents. Be that as it may, because of bandwidth constraints, it is generally impractical to upload large spectral documents (> 50 MB) remotely to MetaboAnalyst. Rather, raw spectra ought to be pre-prepared (peak picked and/or aligned) utilizing locally installed software to create the necessary (and smaller) peak list documents or peak power tables before uploading

to MetaboAnalyst. This pre-handling step can be finished by any number of locally installable, uninhibitedly available MS spectral preparing instruments, for example, MetAlign (Lommen, 2009), OpenMS (Sturm et al., 2008), MZmine

CHAPTER 3: RESULTS

3.1 Survival of Rats

A sum of 32 maturing dark colored Norway rats were utilized for this investigation. Animals were randomly appointed to 4 groups for a 23-week dietary intercession to research the in-vivo impact of OPP on AD initiated by an atherogenic eating routine. At endpoint (week 23), each gathering had lost three animals before the end of the examination because of death or conditions that requested an early euthanization.

3.2 Body weight and diet intake:

No critical contrasts were seen among groups at baseline (week 1) and endpoint (week 23) regarding mean body weight. For count calories consumption, normal eating routine admission (g) every week (the aggregate eating routine admission amid the entire examination time frame/23 weeks) was figured and thought about among four gatherings as opposed to contrasting the benchmark with endpoint due with transforming from the office standard chow eating regimen to exploratory eating methodologies at the gauge. Essentially, the methods for week after week eat less admission were not observed to be huge between among four gatherings.

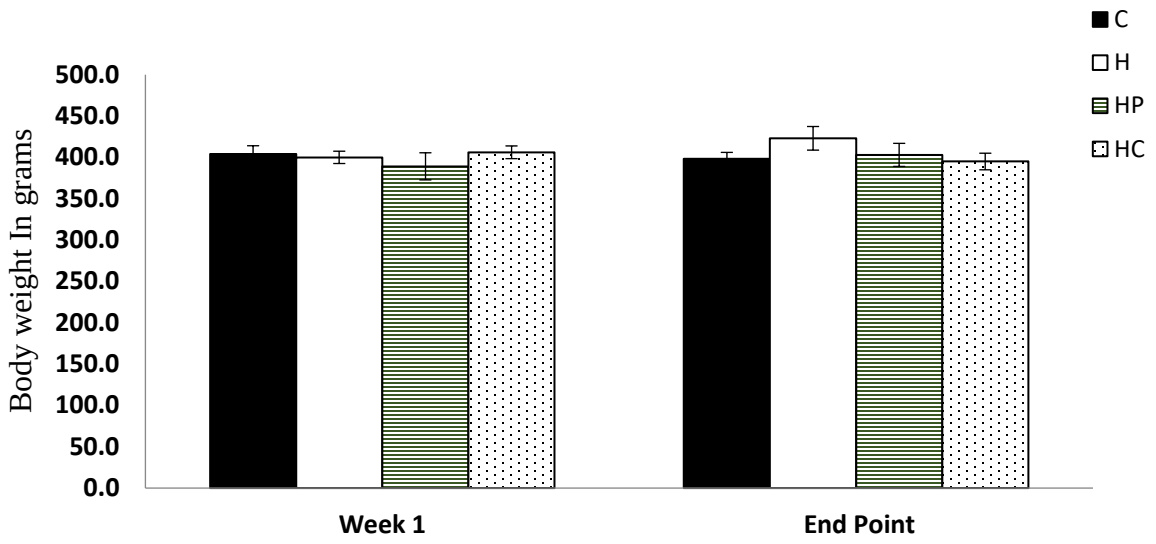


Fig 8: Comparison of mean body weight in Control (C), High cholesterol (H), High cholesterol + 5% OPP (HP) and High cholesterol + 2% curcumin (HC) at baseline (week 1) and endpoint (week 23). Data are expressed as mean±SE. No significant differences between groups observed ($p>0.05$).

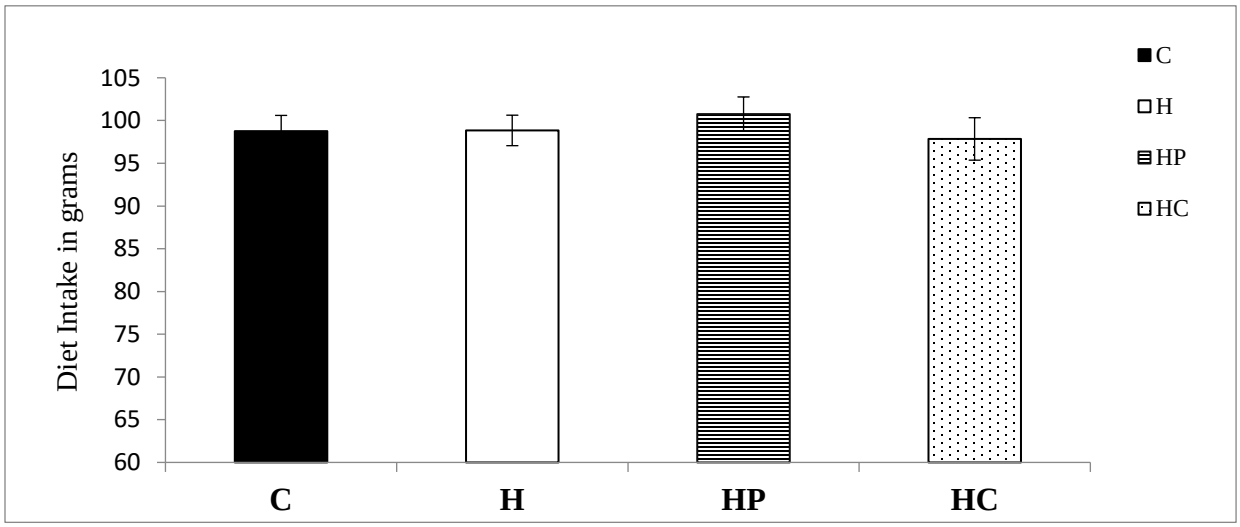


Fig 9: Comparison of mean weekly diet intake in Control (C), High cholesterol (H), High cholesterol + 5% OPP (HP) and High cholesterol + 2% curcumin (HC) over the whole feeding period. Data are expressed as mean±SE. No significant differences between groups observed ($p>0.05$)

3.3 NMR Spectroscopy and Multivariate analysis:

Plasma samples were selected based on the atherogenic diet. A minimum of 750 µl is required for NMR spectroscopy, so we selected samples with at least one aliquot of 1ml. NMR spectroscopy was performed on Varian 600s (at 600 MHZ) and the fid files were processed using ACD software (fig). The processed NMR spectra were digitized and the table of integrals from ACD software was exported to SIMCA P+ software for multivariate statistical analysis. The integrals corresponding to the NMR spectral region from 4.5ppm to 6 ppm were removed from Principal Component Analysis (PCA) as this area contains water peak and other metabolite with exchangeable protons before PCA. PCA score plot showed clustering of one atherogenic diet group from other (fig 12). To observe the clear distinction, the supervised method, OPLS DA plots were plotted.

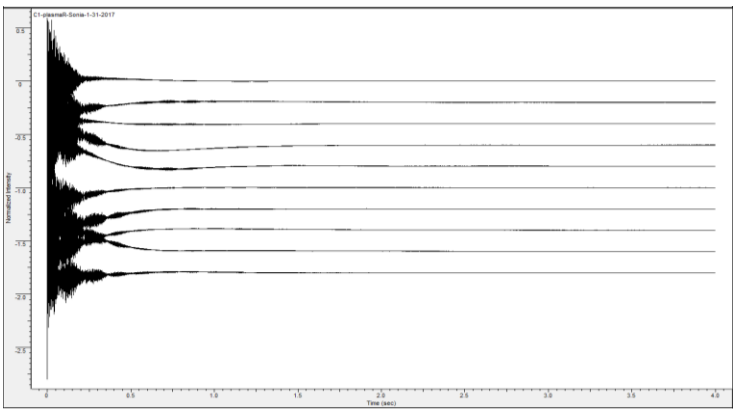


Fig 10a. Fid file

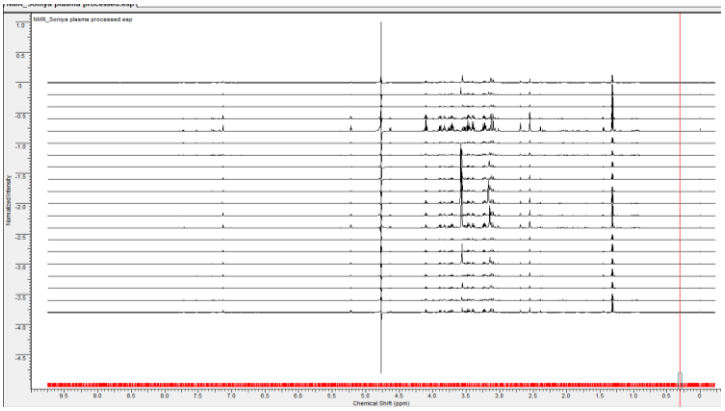


Fig 10.b. Pre-processing of NMR files by ACD software

Specific Aim 1A: To investigate differences in metabolomic profiles of Atherogenic Diet Induced Rat Model of Alzheimer's Disease (AD) and treatment with dietary OPP/ curcumin in Control (C), High cholesterol (H), High cholesterol + 5% OPP (HP) and High cholesterol + 2% curcumin (HC)

Contrasts in the metabolomic profiles of aging rats subjected to various eating methodologies (standard control diet, 2% high cholesterol diet, 2% high cholesterol diet+5% OPP diet, 2% high cholesterol diet+2% curcumin diet) in this examination were assessed utilizing, 1H NMR (proton Nuclear Magnetic Resonance spectroscopy). We aimed to look at possible differences in the plasma metabolomic profiles of different diet groups, using multivariate analysis. The OPLS DA score plot (Fig 13) shows the separation among all the four diet groups.

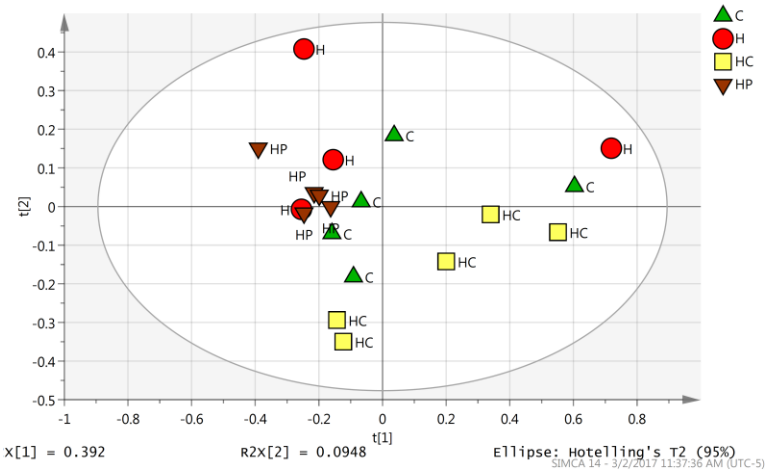
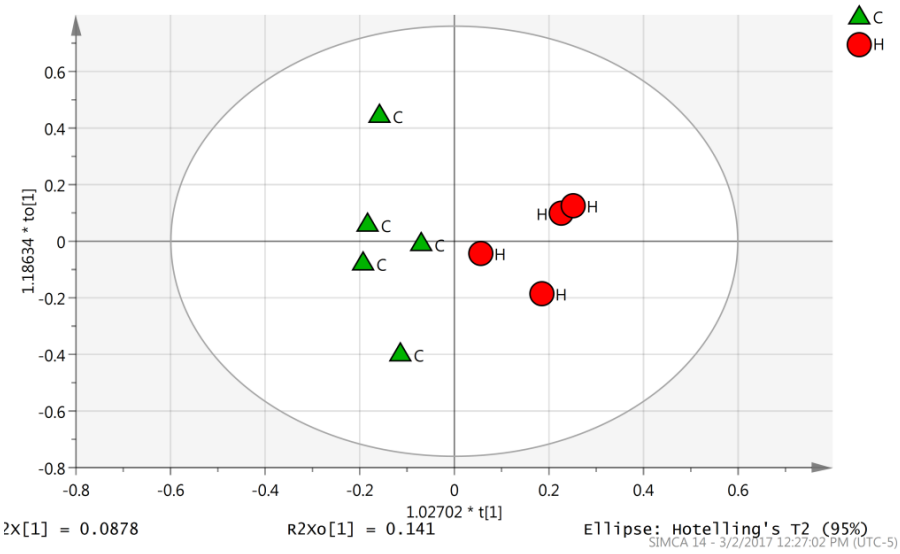


Fig 11: Multivariate analysis of four groups: The supervised method, PLSDA score plot based on plasma ^{1}H NMR spectra of rats of four groups with control, high cholesterol, high cholesterol + curcumin and high cholesterol + OPP

A.



B.

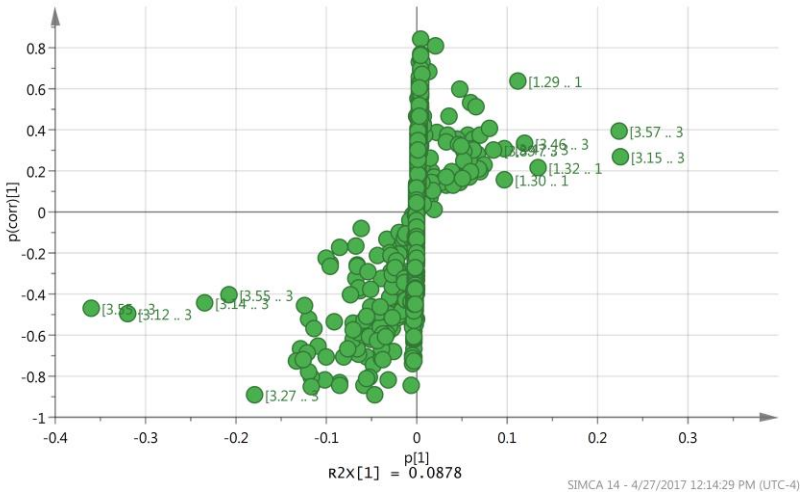
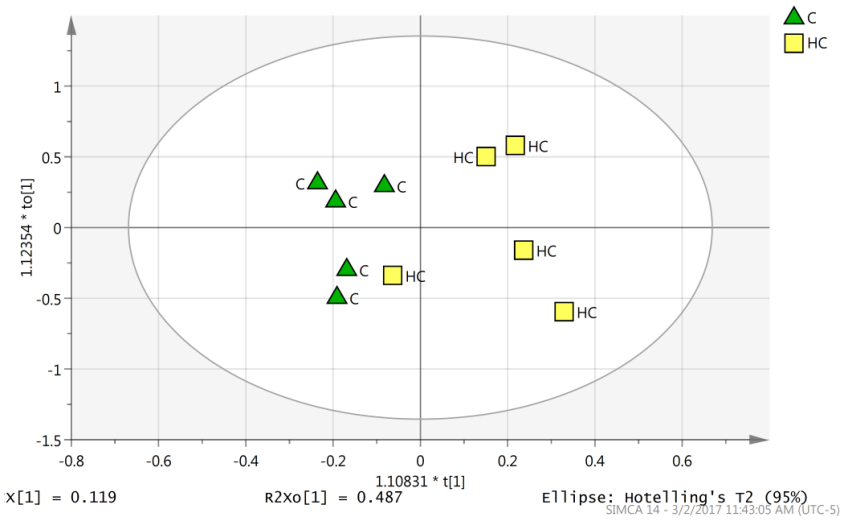


Fig 12A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of Control and high cholesterol group.

Fig 12B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

A.



B.

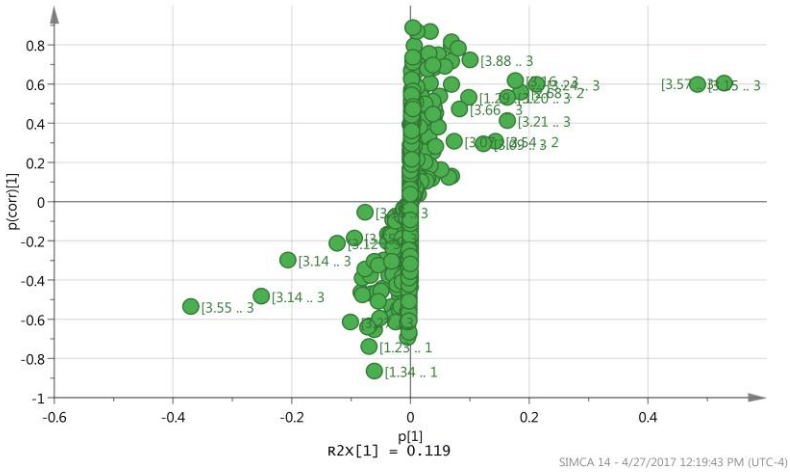
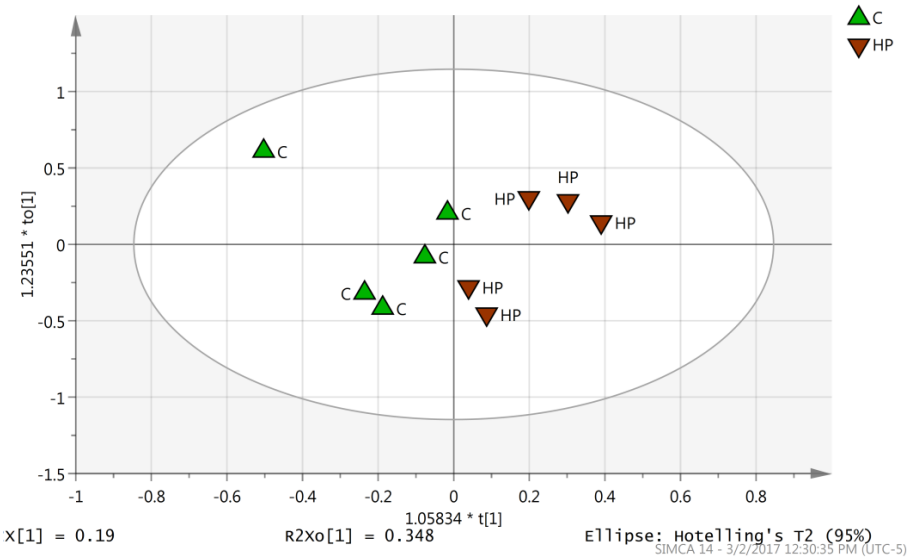


Fig 13A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of Control and high cholesterol + curcumin (Curcumin) group.

Fig 13B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

A.



B.

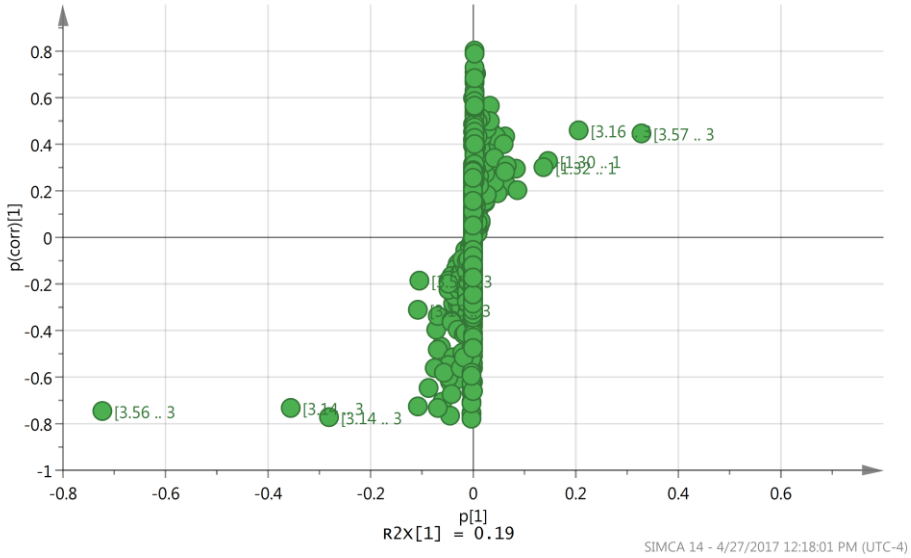
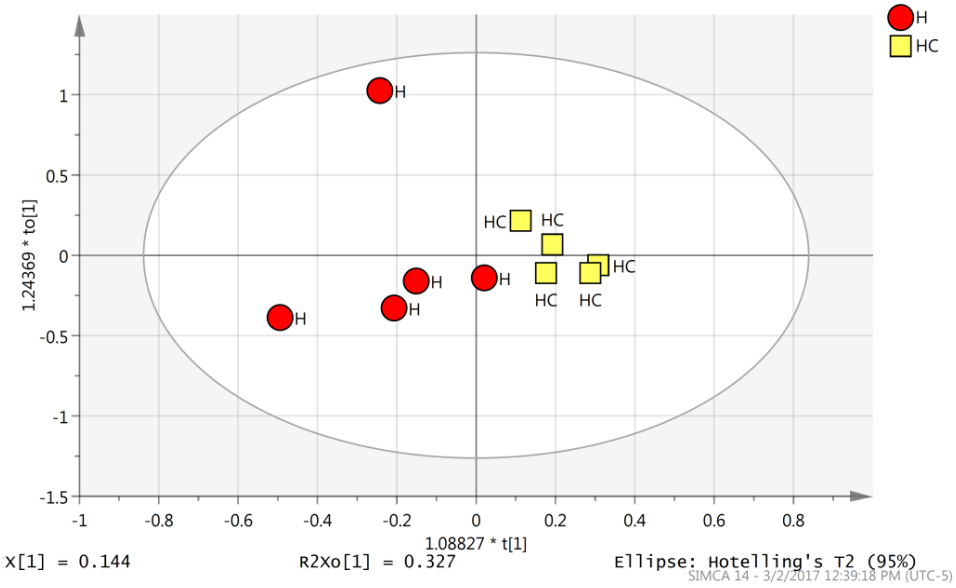


Fig 14A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of Control and high cholesterol + OPP (OPP) group.

Fig 14B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

A.



B.

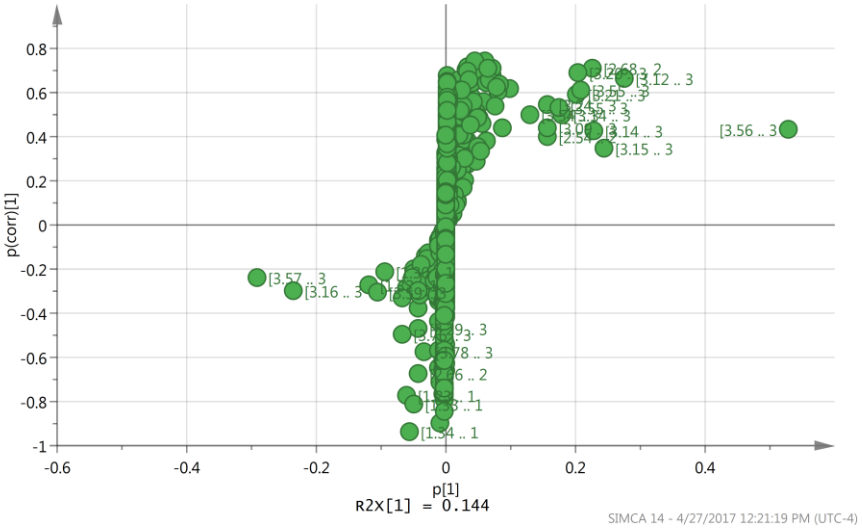
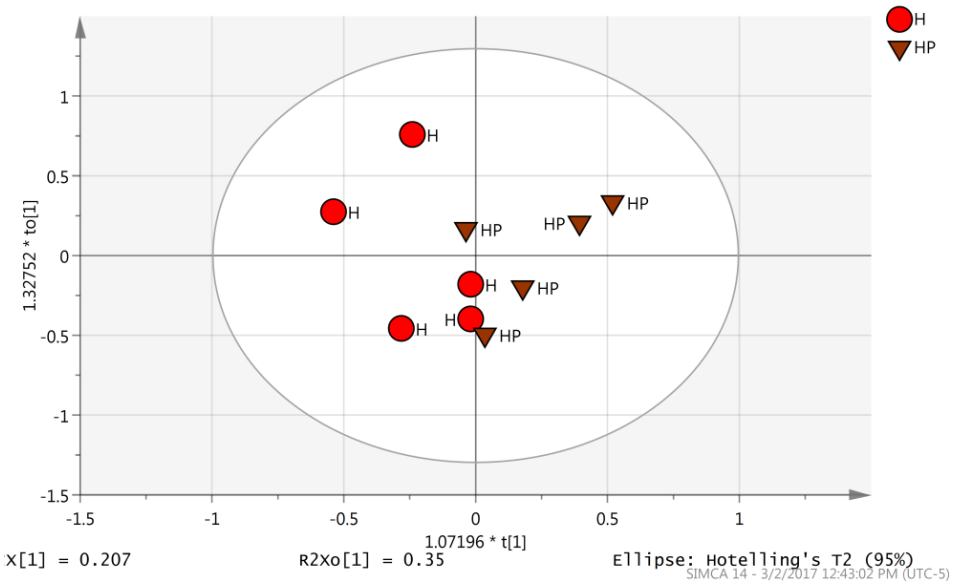


Fig 15A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of High Cholesterol and high cholesterol + Curcumin (Curcumin) group.

Fig 15B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

A.



B.

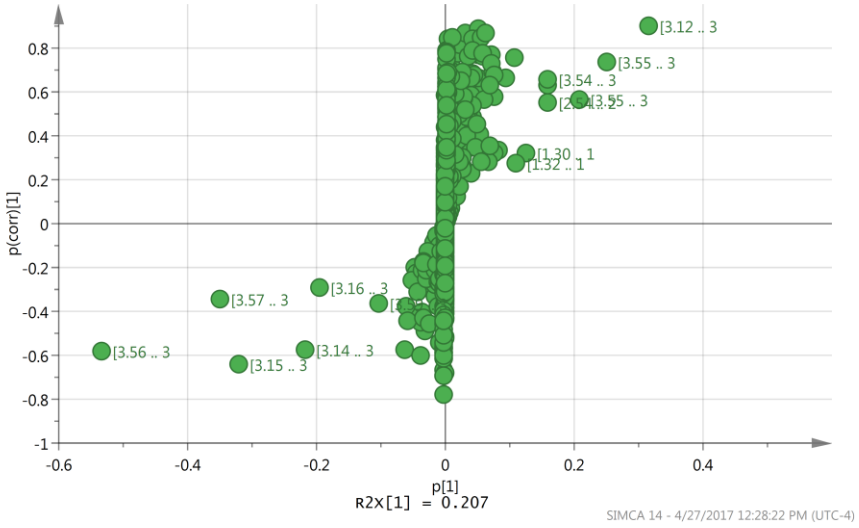
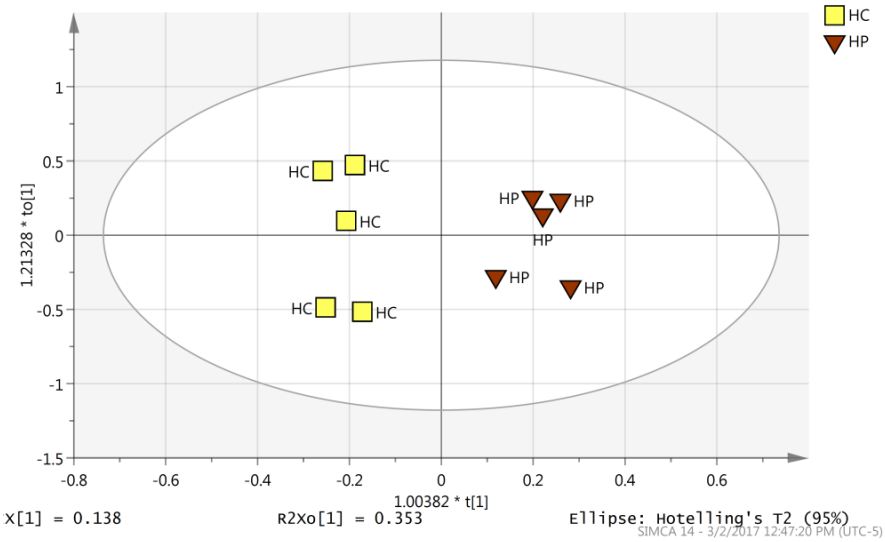


Fig 16A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of High Cholesterol and high cholesterol + OPP (OPP) group.

Fig 16B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

A.



B.

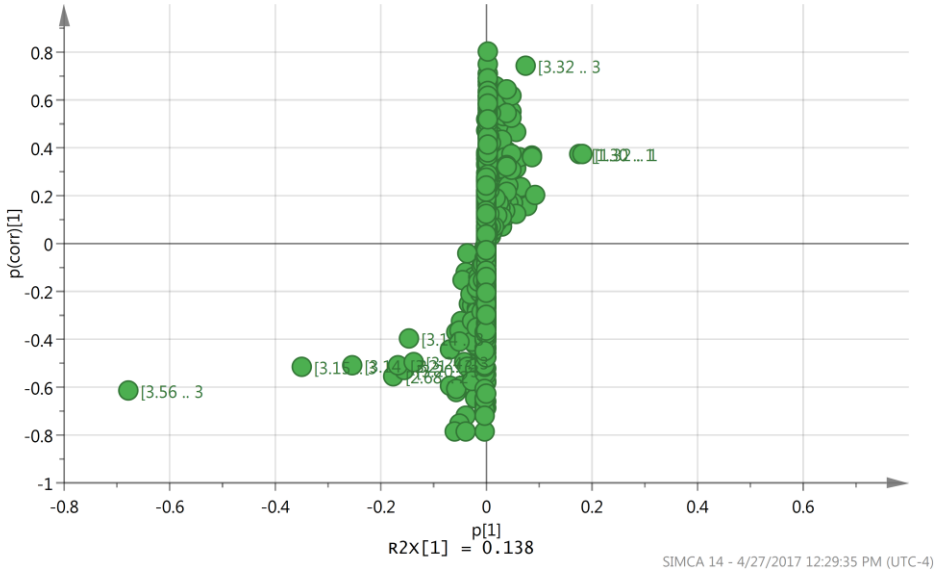
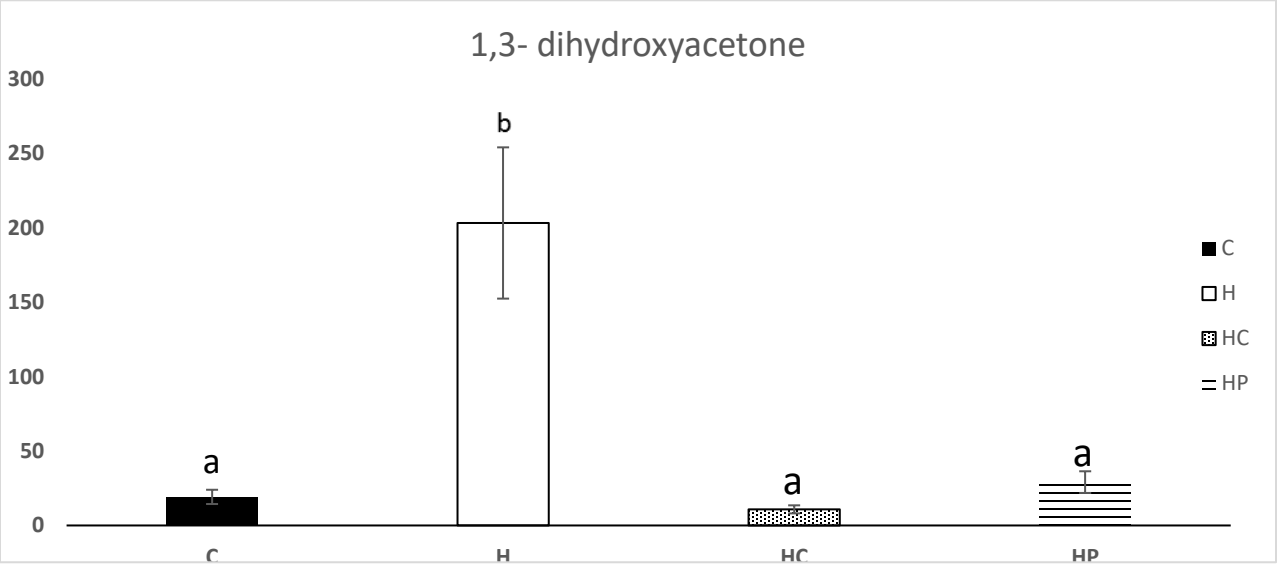


Fig 17A: Multivariate analysis of two groups: The OPLS DA score plot based on ^{1}H NMR spectra of rats of High Cholesterol + Curcumin (Curcumin) and high cholesterol + OPP (OPP) groups

Fig 17B: Multivariate analysis of two groups: The loading plot depicting the spectral regions responsible for separation between two diet groups at different ppm.

Specific Aim 1B: To identify and quantify metabolites responsible for the differences in Metabolomic Profiles of Control and treated groups.



A : C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

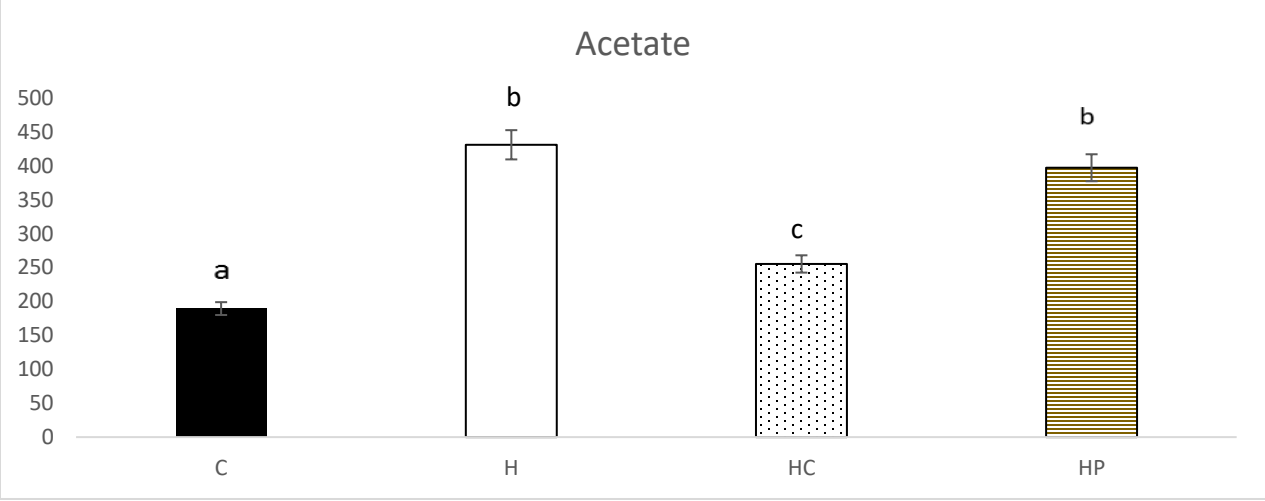


Fig B: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

a & c are significantly different from each other ($p<0.05$)

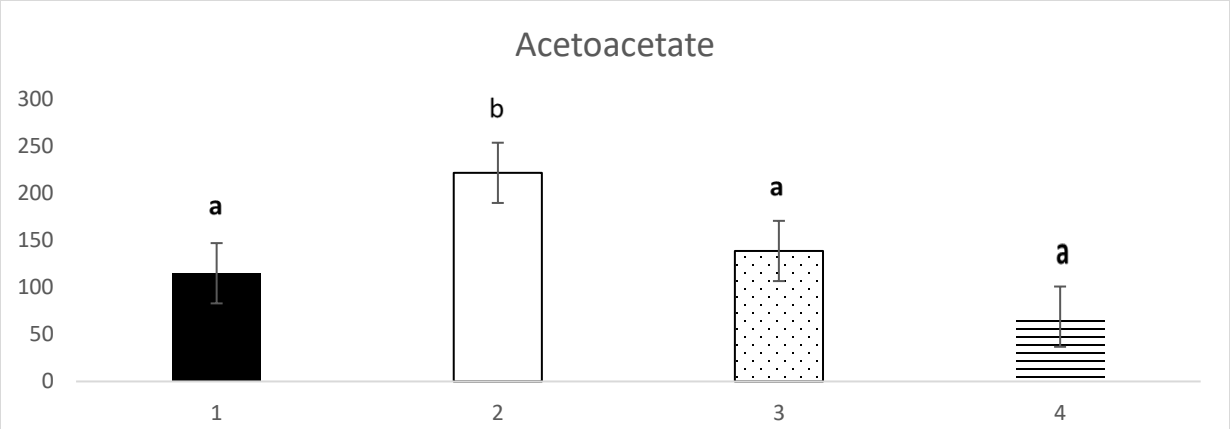


Fig C: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

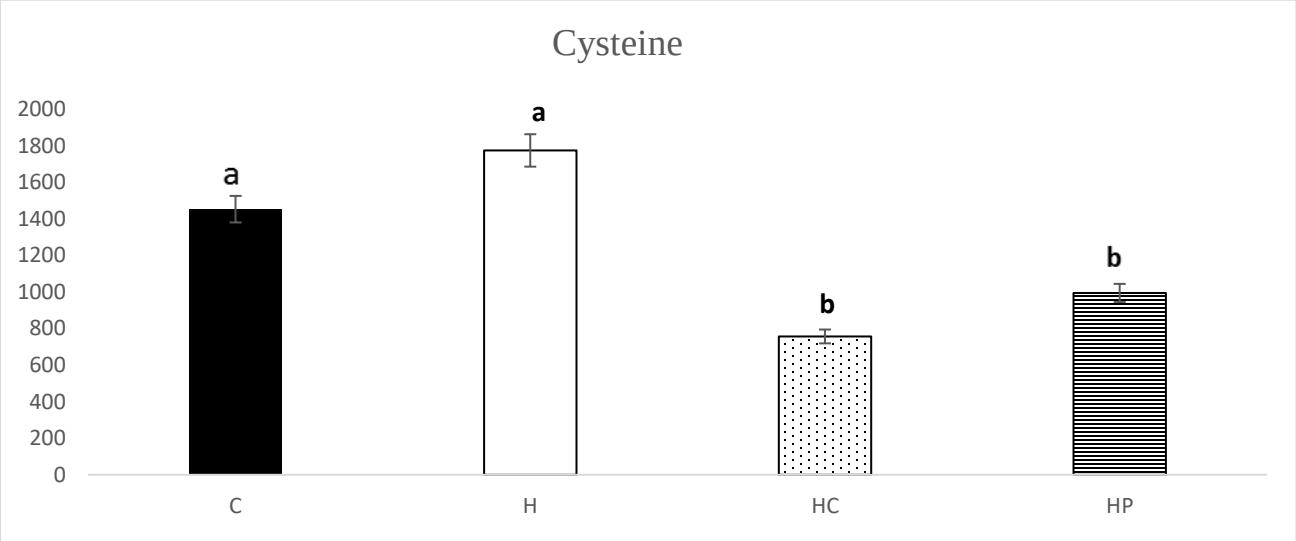


Fig D: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

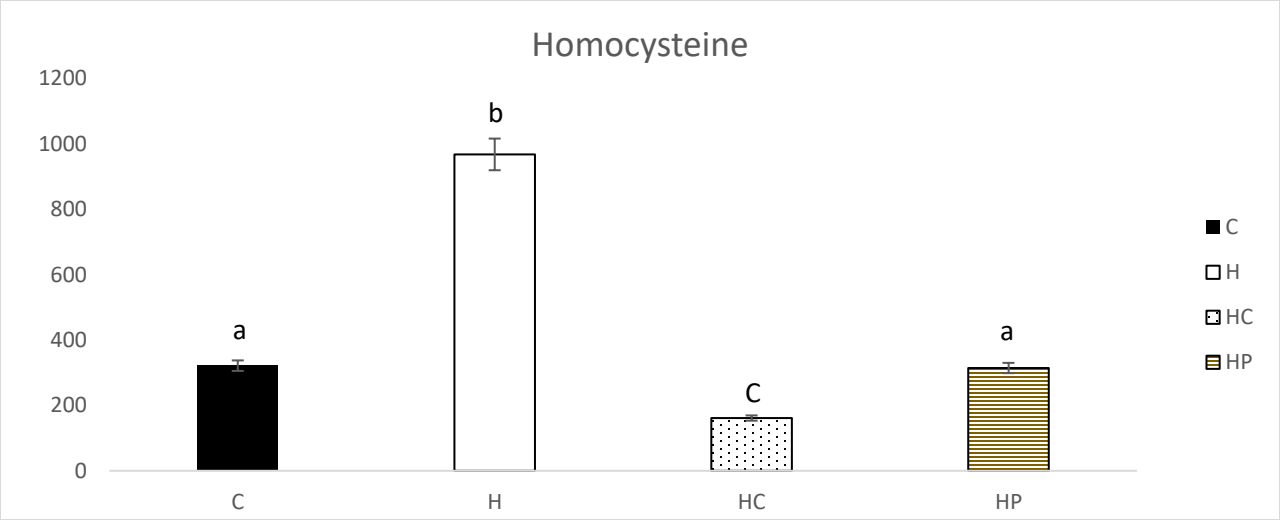


Fig E: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

c is significantly different from a & b ($p<0.05$)

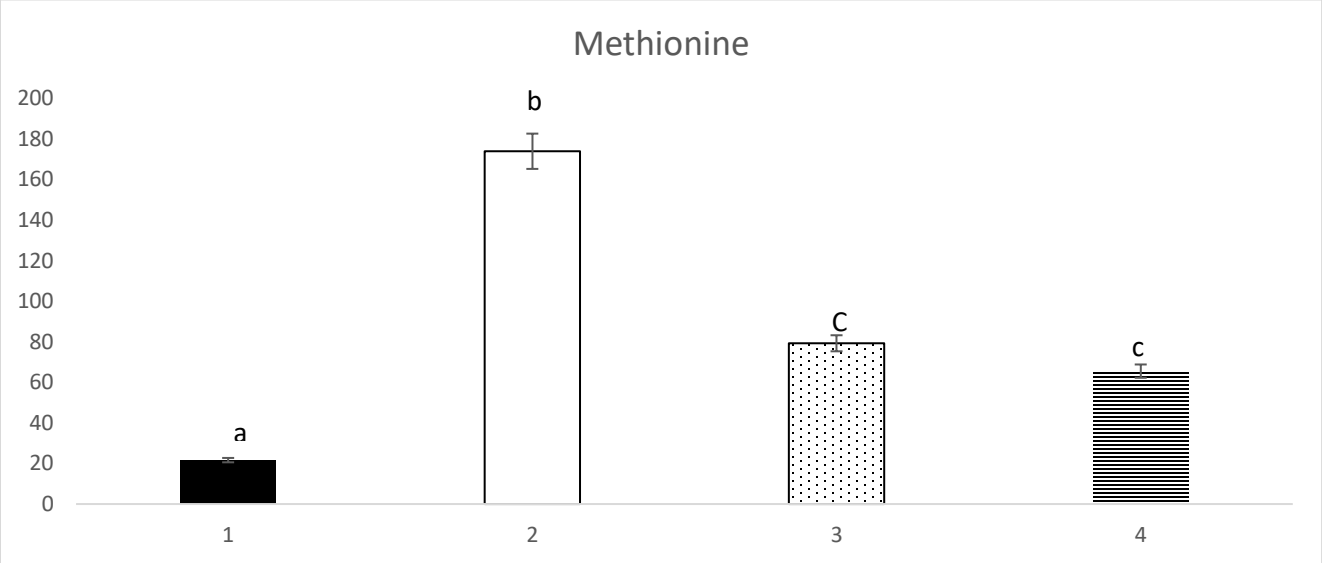


Fig F: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

c is significantly different from a & b ($p<0.05$)

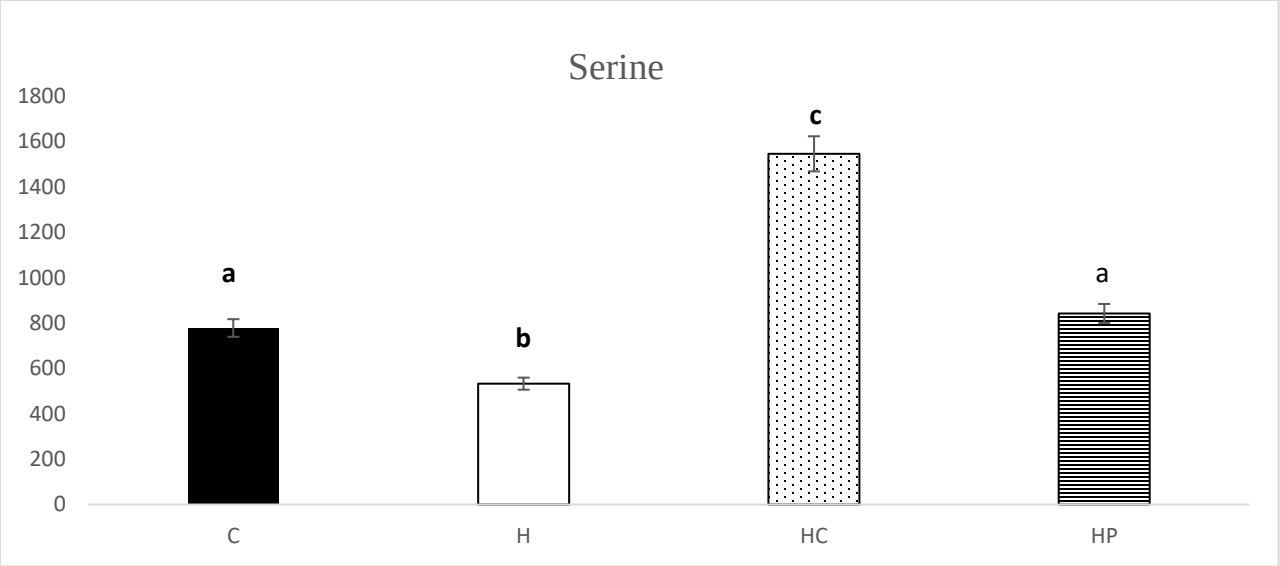


Fig G: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

c is significantly different from a & b ($p<0.05$)

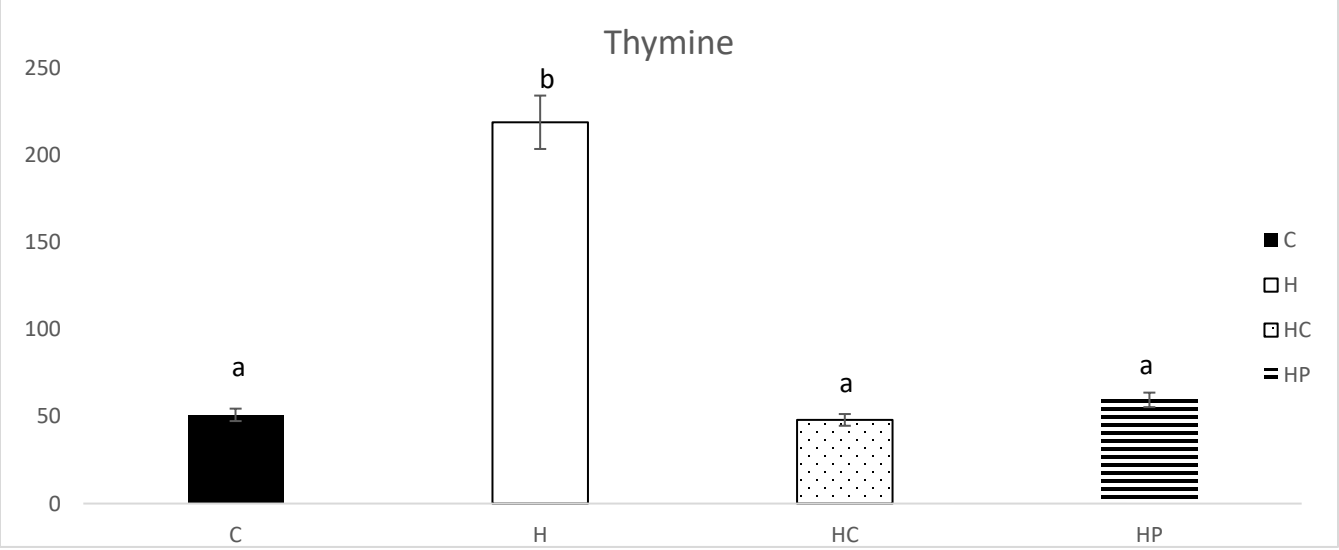


Fig H: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$)

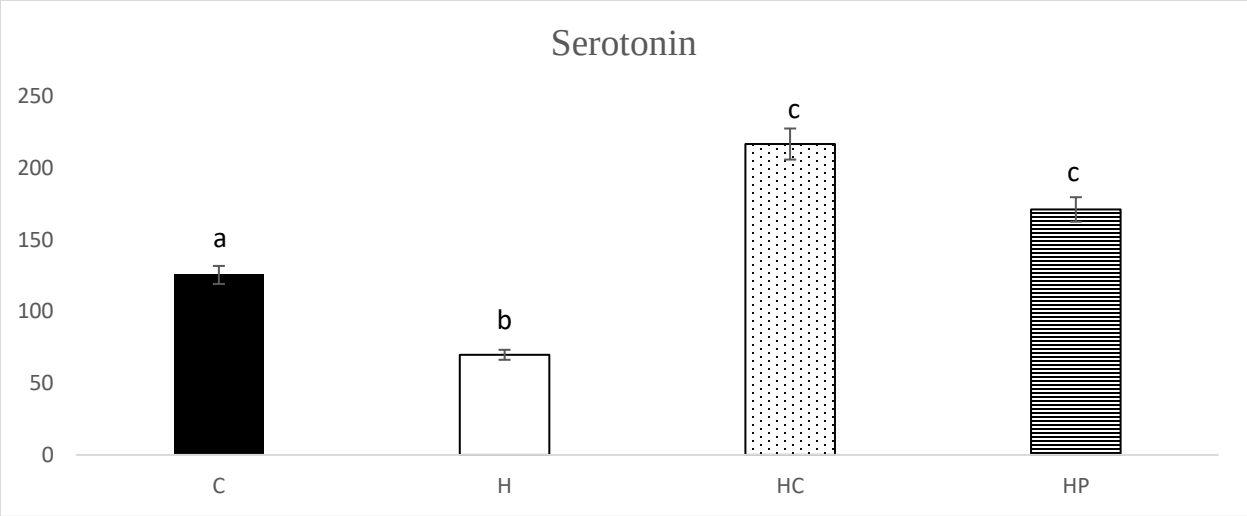


Fig I: C – Control H- High Cholesterol, HC – High cholesterol + Curcumin, HP – High Cholesterol +OPP

a & b are significantly different from each other ($p<0.05$) , c is significantly different from a & b ($p<0.05$)

Fig 18: Selected plasma metabolites associated with the diet effect found in Homocysteine metabolism by the pathway analysis

Table 3. Changes in the metabolomic profile due to the diet effect

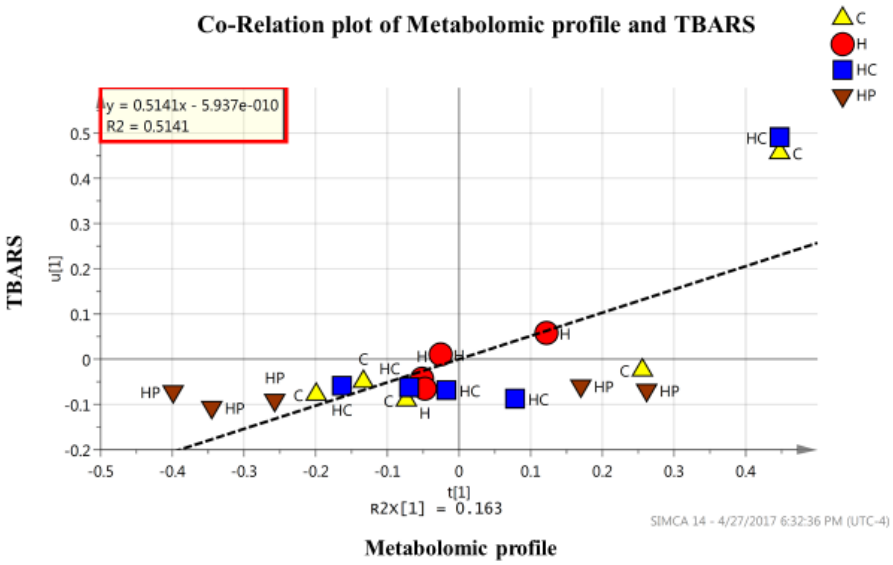
Compounds	Chemical shift	P value	Fisher's LSD post-hoc
2-Oxoglutarate	4.0 2.3 2.2 2.0 1.8	<0.01	Control - Curcumin; Control - OPP; HighCho - Curcumin; HighCho - OPP
Melatonin		<0.01	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
4-Hydroxyphenylacetate	7.2 6.9 3.4	<0.01	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP

Catechol	6.9 7.0	<0.01	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
dTTP	1.9 2.4 4.2 4.6 6.3 7.7	<0.01	Curcumin - Control; OPP - Control; Curcumin - HighCho
Phenol	7.0 7.3 6.9	<0.01	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
Acetoacetate	2.3 3.4	0.02	HighCho - Control; HighCho - Curcumin; HighCho - OPP
Riboflavin	2.5 2.6 3.7 3.9 4.0 4.4 5.0 8.0	0.02	Control - HighCho; Control - OPP
Taurine	3.2 3.4	0.02	Control - Curcumin; Control - HighCho; Control - OPP
Allantoin	8.0 7.3 6.0 5.4	0.02	HighCho - Control; HighCho - Curcumin; HighCho - OPP
Citrate	2.7 2.5	0.03	Control - Curcumin; Control - OPP
Serotonin	3.1 3.3 6.9 7.1 7.3 7.4 10	0.03	Curcumin - Control; Curcumin - HighCho
Homoserine	2.0 2.2 3.8 3.9	0.03	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
Phthalate	7.5	0.03	Curcumin - Control; Curcumin - OPP
Theophylline	8.0 3.6 3.4	0.03	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
Tyrosine	7.2 6.9 3.9 3.2 3	0.03	Curcumin - Control; Curcumin - HighCho
Ethanolamine	3.1 3.8	0.03	Control - Curcumin; HighCho - Curcumin; HighCho - OPP

N-Phenylacetylglycine	3.7 7.3 7.4 7.9	0.04	Curcumin - Control; Curcumin - HighCho; Curcumin - OPP
Homocysteine	2.2 2.3 2.8 2.9 3.9	0.04	HighCho - Control; HighCho - Curcumin
3-hydroxybutyrate	1.2 2.3 2.4 4.1	0.04	HighCho - Control; HighCho - Curcumin; HighCho - OPP
Trimethylamine	2.9	0.04	HighCho - Curcumin; HighCho - OPP
Gentisate	6.8 7.0 7.3	0.04	HighCho - Control; HighCho - Curcumin; HighCho - OPP
Serine	4.0 3.9 3.8	0.04	HighCho - Control

The relationship between plasma metabolite profiles with some of the variables that is related to the AD progression such as hippocampal βamyloid 42 concentration, brain oxidative stress and peripheral inflammation(Il-6) were also performed by regression analysis. As seen from the fig. 29, OPLS regression shows moderate correlation (R^2=0.5276) between peripheral inflammation and plasma NMR metabolomic profile. Similarly, the OPLS regression plot between hippocampus βAmyloid 42 concentration (fig.30) with plasma ^{1}H NMR profiles of the four groups at end time point shows a moderate correlation too (R^2= 0.5765). The S-plot obtained from OPLS model at endpoint indicates the regions of the metabolites that are respectively positively and negatively correlated with the IL-6. There is a moderate correlation (R^2= 0.5141) between brain oxidative stress level (MDA) with plasma 1H NMR profiles of the four groups at end time point (fig. 30). The S-plot shows the respective positive and negative co relation between brain oxidative stress level (MDA) and plasma metabolomic profile.

A.



B.

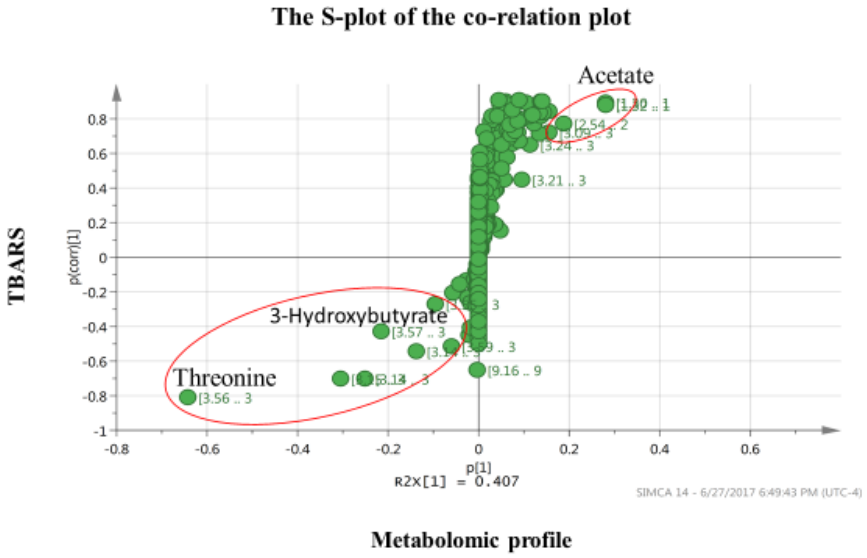
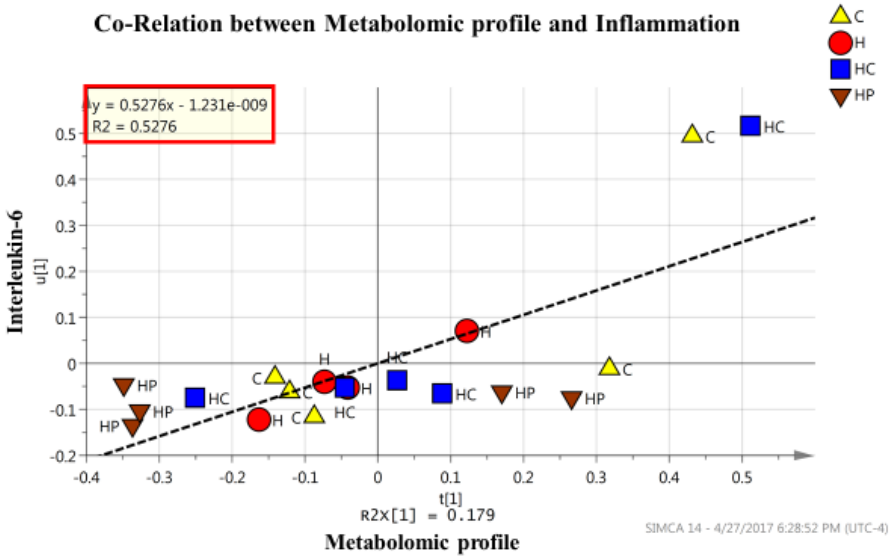


Fig.19: OPLS regression of brain oxidative stress level (MDA) with plasma ^{1}H NMR profiles of the four groups at end time point. (**A**) OPLS score shows a moderate correlation (R^2=0.5141) at endpoint (week 22). **(B)** S-plot obtained from OPLS model. The circle on the upper right side includes the regions of metabolites in the spectra that is correlated with higher MDA level; Regions containing regions of the metabolites are circled at lower left side are correlated lower MDA level.



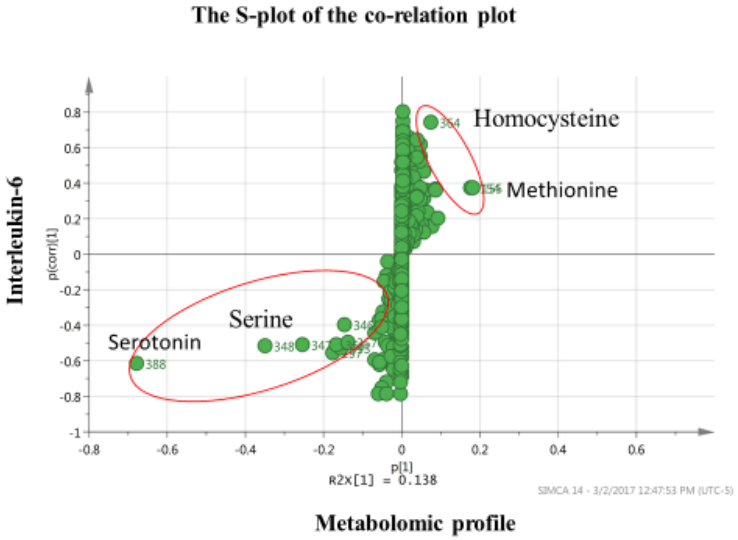
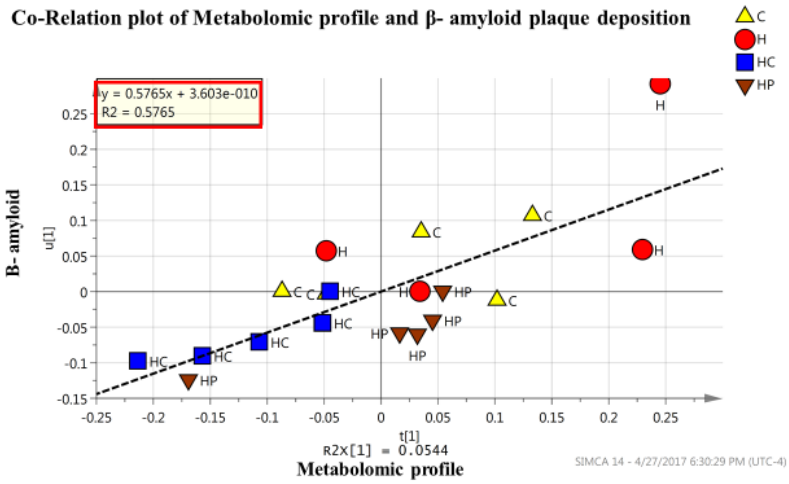


Fig 20. OPLS regression of plasma inflammation level (IL-6) with plasma ^{1}H NMR profiles of the four groups at end time point. (**A)** OPLS score shows a moderate correlation (R^2=0.5276) at (week 22). **(B)** S-plot obtained from OPLS model. The circle on the upper right side includes the regions of metabolites in the spectra that is correlated with higher higher Il-6 level; Regions containing regions of the metabolites are circled at lower left side are correlated lower Il-6 level.

A.



B.

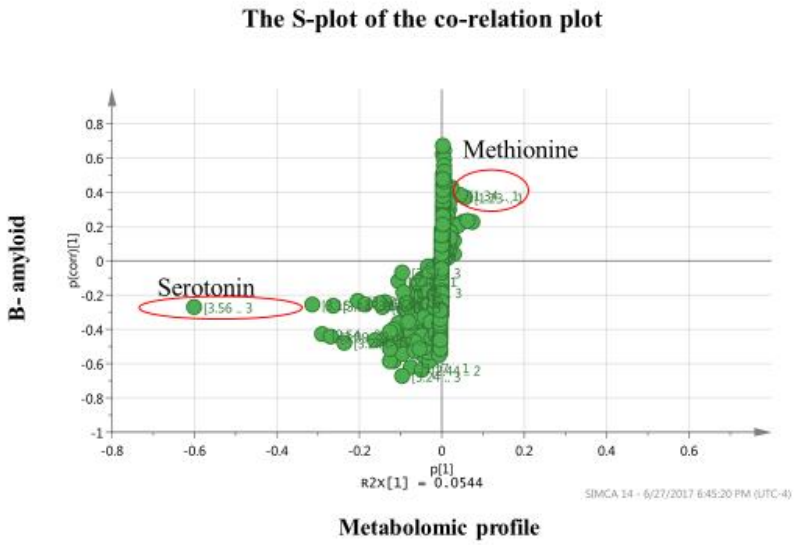


Fig 21. OPLS regression of hippocampus βAmyloid 42 concentration with plasma ^{1}H NMR profiles of the four groups at end time point. (A) OPLS score shows a moderate correlation (R^2=0.5765) at endpoint (week 22). **(B)** S-plot obtained from OPLS model. The circle on the upper right side includes the regions of metabolites in the spectra that is correlated with higher βAmyloid 42 concentration; Regions containing regions of the metabolites are circled at lower left side are correlated lower βAmyloid 42 concentration.

Specific Aim 2: To identify the pathways involved in the differences in the plasma metabolites markers.

Pathway analysis was performed using the software Metaboanalyst 3.0. The. exl file was converted to .csv file, as the software can accept only comma separated file. A .csv or .txt document is typically utilized when the entire data set has already been pre-prepared to a tabular shape, for example, a compound concentration table, a spectral bin table, or a peak force table. The compress document format is typically used to upload different MS spectra or numerous peak list records. The file is further processed and normalized to obtain the pathway. Fig. 32 depicts the impacted pathway and their network analysis. The significant pathway observed in this analysis is Cysteine-Methionine Metabolism and Glycine, serine, taurine Metabolism. The fig 23-25 depicts the changes in the metabolites leading to the significant change in the Cysteine-Methionine Metabolism.

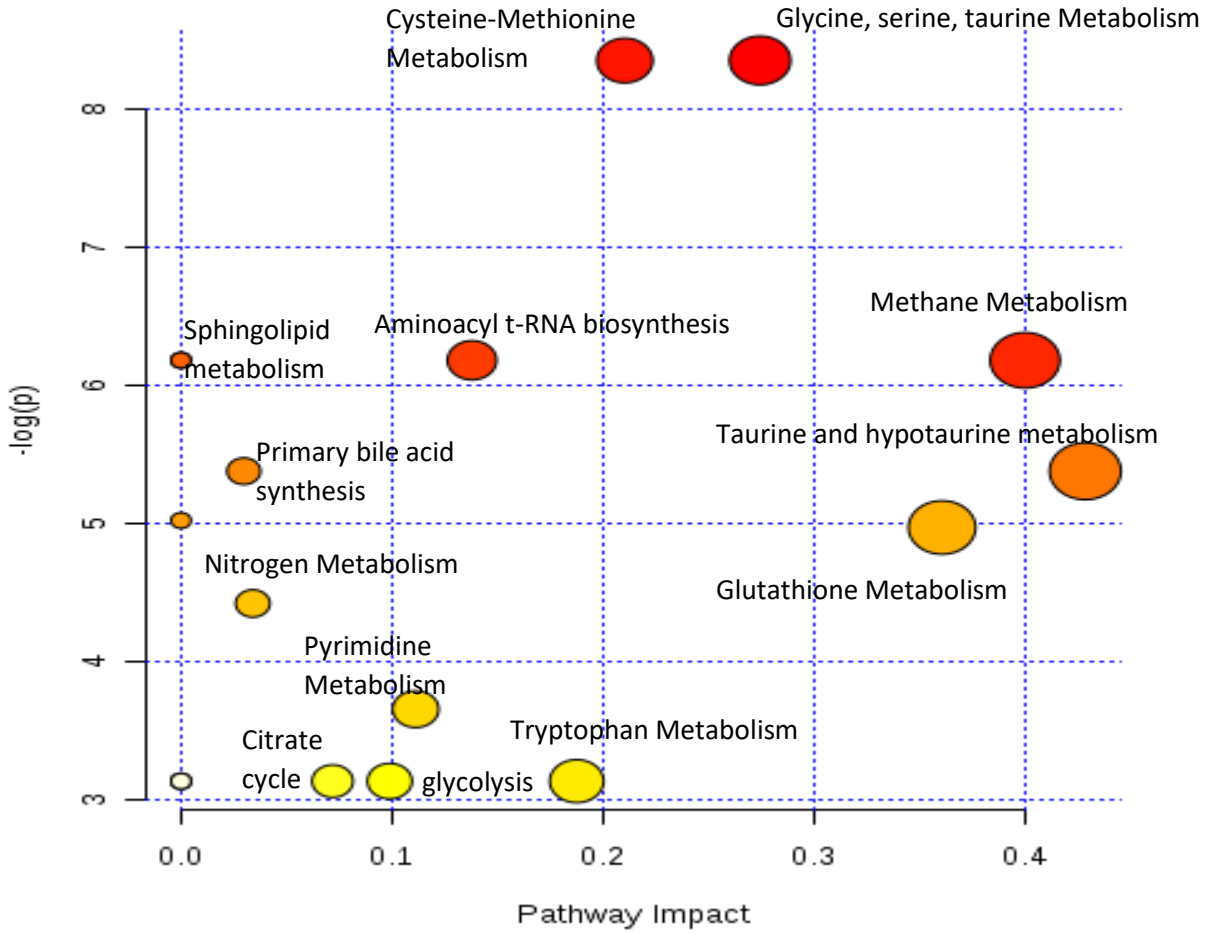


Fig 22. Pathway analysis. MetaboAnalyst 3.0 output illustrating the most predominant metabolic pathways that correspond to the significant metabolites changed in the plasma metabolomic profiles. The larger a circle and higher on the y axis, the higher impact of pathway.

CHAPTER 4: DISCUSSION

Homocysteine is a non-protein α-amino corrosive. It is a homologue of the amino corrosive cysteine, differing by an extra methylene connect. It is biosynthesized from methionine by the expulsion of its terminal Cε methyl gathering.

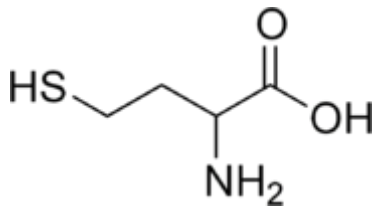


Fig 23: Structure of Homocysteine[28]

Mechanisms by which HCY influences AD are obscure. Be that as it may, deleterious mechanisms involving HCY have been studied with regards to cardiovascular disease. Because of a growing acknowledgment that cerebrovascular disease may advance AD, ideas taken from studies of HCY and coronary illness research are being stretched out to the brain. For instance, plasma HCY might be specifically harmful to vascular endothelial cells or induce their dysfunction, leading to loss of blood-brain-barrier work and changed generation of nitric oxide. What's more, if HCY crosses the blood-brain barrier or is released by cells in the brain, it would act be able to as a powerful neurotoxin HCY levels anticipated the incidence of AD and different dementias even after adjustment for known risk factors of age, sex, and hereditary type of apoE. This work is essential for several reasons. First, this study is prospective in nature, and results have prescient esteem. A substantial number of individuals were included, and, notwithstanding HCY, metabolic parameters identified with HCY were evaluated. Second, the quantity of epidemiologic studies addressing the part of HCY in AD and different dementias is few, and further work, such as that done by Seshadri and colleagues, is required using all around defined populations. Finally, because levels of homocysteine can be balanced by dietary folate, this study pinpoints a potential focus for AD counteractive action. Such neurotoxic effects might be because of direct interaction of HCY with plasma

film components, or because of intracellular aggregation of S-adenosyl-homocysteine. This last metabolite inhibits methylation of catechol substrates resulting in the era of oxyradicals and other synthetically responsive products that are cytotoxic. In general, HCY may harm brain tissue through different pathways, which are yet unidentified[29].

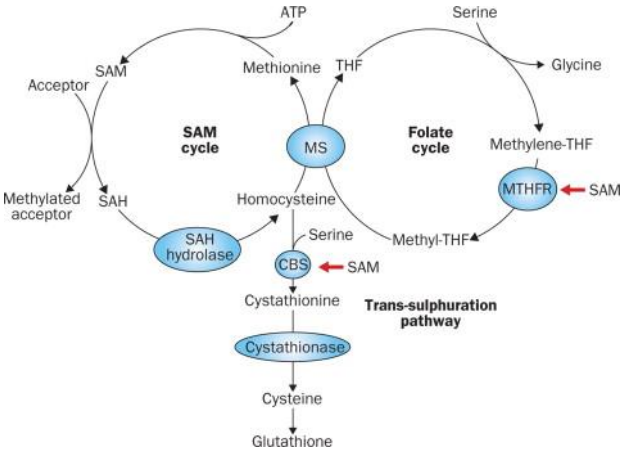


Fig 24: Cysteine-Methionine Pathway[30]

The research data till now suggests that AD is the most common form of neurodegenerative disorder. In its sporadic shape, AD results from the blend of hereditary components with various epigenetic occasions. The diagnosis of AD is usually based on the symptoms that occurs after the age of 65. The research shows some neurotic components that have recently been found in the brain before the manifestations show up, which make the patient lose the most obvious opportunity for early determination and treatment of AD. Therefore, animal models have been produced and connected to illustrate the AD obsessive movement and systems. There is an expanding consciousness that way of life particularly a high fat/cholesterol slim down is assuming a negative part to cardiovascular capacity, tumor rates, and cognitive function. A study by Granholm AC, et. Al revealed that diet rich in cholesterol and/or saturated fats is deleterious to cognitive function.

They fed 16-month old rats, cholesterol (2%) and saturated fat (hydrogenated coconut oil, Sat Fat 10%) diet for two months to investigate the impacts on memory of the rats, thus suggesting, cholesterol can immensely impair memory[31]. The lipid profiles revealed elevated total cholesterol levels. In our present study, when we performed multivariate analysis using SIMCA p+ software, it exhibited the separation between different diet groups, at 3.14, 3.15, 3.32, 3.55, 3.56, 3.57 ppm. The further analysis was done to identify and quantify these metabolites involved using CHENOMX and t-test to obtain the significantly different metabolites. Snowden, et al., applied untargeted metabolomics to the brain tissue collected by autopsy from three different groups, AD, control and asymptomatic Alzheimer's disease to study difference in brain metabolite levels. They analyzed the center frontal gyrus, inferior temporal gyrus and the cerebellum (CB), which is generally saved of established AD pathology. They found that unsaturated fatty acids were significantly decreased in Alzheimer's brains when compared to brains from healthy patients and DHA, increased with the progression of the disease[32]. Similarly, our results (fig 20) showed increased level of 1,3-Dihydroxyacetone in the high cholesterol fed diet, thus indicating the dementia. A current report announced by Schernthaner, that ketoacidosis autonomously instigated changes in pro inflammatory cytokines, oxidative stress and CVD as large amounts of circulating IL-6 and TNF-α were lifted in hyperketonemic diabetics yet not in ordinary diabetic patients[33]. Ketones such as acetoacetate (AA) has been reported to increase cellular lipid peroxidation resulting from oxygen radical production by AA or other effects of AA on enzymes or signal transduction pathways that in turn lead to elevated oxidative stress. The oxygen radicals generated by the ketone body acetoacetate can exert cytotoxic effect by causing peroxidation of membrane phospholipids and the resulting accumulation of peroxidation products such as malondialdehyde (MDA). These products have been known to cross-link membrane components and result in altered

membrane permeability and an ultimate cellular dysfunction[34]. The ketones such as acetoacetate and acetate were also found to be high in our high cholesterol fed diet animals (fig 21, 22) thus reflecting high oxidative stress. The level of these ketones was significantly reduced by curcumin and OPP fed diet. Thus, demonstrating the antioxidant and anti-inflammatory effect on the ketones in the plasma. This is in accordance with another study by Li et al. [35]who showed that curcumin administration significantly reduced ketone body levels in mice fed high fat diet. In conclusion, supplementation with OPP and curcumin reduces oxidation-induced ketone generation and attenuates the atherosclerosis events in rats fed a high cholesterol diet. One of the pathway with high significance and impact in rat's plasma profile is Homocysteine metabolism. Some metabolites from its metabolism were significantly higher in the cholesterol fed animals as compared to the control group. Treatment with curcumin and OPP brought down the concentration of these metabolites significantly and closer to the control levels. Significant effects were observed in serine, cysteine, methionine in the curcumin diet groups A scope of mechanisms having been proposed for the connection between elevated Homocysteine (Hcy) and AD and preclinical investigations demonstrate that hyperhomocysteinemia, initiated by hereditary control or by B-vitamin insufficiency, causes referred to signs of AD, for example, aggregation of amyloid-β peptide[36]. A post-mortem examination demonstrated a clear association between Hcy levels and neurofibrillary tangles, a known sign of AD[37]. Amyloid plaque arrangement is believed to be an imperative occasion in the etiology of AD and there is affirmation that raised levels of Hcy can affect the plaque development by lessening the clearing rate of amyloid-β in the mind of mice and can induce oxidative stress[38]. Amyloid-β levels increased in rats after infusion of Hcy into their mind along with loss in spatial memory[39]. In accordance, we found significantly high level of homocysteine in the high cholesterol fed diet animal thus indicating Alzheimer's Disease. But, on

the other side, the level of homocysteine was significantly decreased in the High Cholesterol + Curcumin fed diet animal (fig 24).

To verify the results further, the correlation plot was plotted between the plasma metabolomic profile and lipid oxidation indicator, inflammation marker (IL-6) and amyloid plaque deposition. All the correlation plot (Fig 29, 30, 31) showed more than 50% significance. Thus, suggesting that the data is strongly correlated.

In conclusion, curcumin and OPP have anti-inflammatory and anti-oxidative effect on the atherogenic diet induced rat models. Observations from this study can be further investigated using larger scale animal studies that may lead to a pilot human study. Some findings from the plasma metabolomic profiles might provide a potential pharmaceutical / nutraceutical target for the future AD treatment.

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ABSTRACT

THE EFFECT OF OIL PALM PHENOLICS (OPP) AND CURCUMIN ON PLASMA METABOLOMIC PROFILE IN ATHEROGENIC DIET INDUCED RAT MODEL OF ALZHEIMER'S DISEASE (AD)

by

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Alzheimer's disease (AD) is the most widely recognized reason for dementia in the aging population. It is described by intellectual decay and deposition of ß-amyloid plaques in the hippocampus. It has been demonstrated that hypercholesterolemia incited by elevated cholesterol abstain from food is related with AD advancement. Expanded level of oxidative stress has additionally been seen in AD patients. An essential methodology to treat or postpone the disability depends on dietary change, utilizing nourishment supplements. OPP, a water-soluble fraction from oil palm fruit, rich in phenolics has been found to possess significant antioxidant activities. Curcumin, a polyphenol extricated from the plant Curcuma longa, has demonstrated its remedial advantages in Alzheimer's ailment and was utilized as a positive control. Our results demonstrated the dietary cholesterol actuated hypercholesterolemia which expanded AD-like pathological changes in matured rats including β-amyloid amassing and psychological decrease. OPP & curcumin attenuate the process of AD for their antioxidant and anti-inflammatory effects by improving these pathological changes. acquire the spectrum of samples. Multivariate analysis software, SIMCA-P+, was applied to demonstrate the differences in plasma 1H NMR profiles among the groups. Partial least Squares (PLS-DA) score plots showed clear separation among all four groups indicating differences in their metabolomics profiles at the end point. OPLS regression analysis gave significant correlations between the urinary metabolomic profiles and the β amyloid

burden. The metabolites responsible for the differences in the metabolomic profile among groups were then quantified using CHENOMX NMR metabolite database. Some metabolites from the homocysteine metabolism pathway were significantly altered in the cholesterol fed group (H) as compared to the treatment groups (HP, HC). Treatment with curcumin (HC) or OPP (HP) modulated the concentration of these metabolites closer to the control levels. This pathway has been shown to be perturbed in neurodegenerative diseases. Taken together, curcumin exhibited a potential therapeutic effect in high cholesterol diet induced AD. Moreover, specific plasma metabolites may serve as non-invasive biomarkers for progression of neurodegenerative diseases including AD.

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