Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2863
DOI URL: http://dx.doi.org/10.21474/IJAR01/2863
ISSN NO. 2320-5407

RESEARCH ARTICLE

STRUCTURAL VALIDATION AND HOMOLOGY MODELING OF DIFFERENTIALLY EXPRESSED PROTEINS IN *RATTUS NORVERGICUS* INDUCED BY BISPHENOL A AND PROBIOTIC TREATEMENT

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Manuscript Info

Manuscript History

Received: 21 November 2016
Final Accepted: 21 December 2016
Published: January 2017

Key words:-
Homology Modeling, Bioinformatics, Bisphenol stressed, Bisphenol and probiotic treated proteins.

Abstract

The aim of the present study was to apply Bio informatics tools to the proteins expressed in alteration with Bisphenol A**.** The structure-based computational methods are needed to help, identify and characterize protein-protein complexes and their function. Differentially expressed Proteins were Tryptic digested and were analyzed by MALDI-TOF to identify peptide masses afterward used for MS/MS**.** For individual proteins, the most successful technique is homology modeling. Based on their mass to charge ratio, the expressed proteins sequences were collected from Mascot search data. The sequences were analysed with the help of Phyre-2 server, RasMol version 2.6 software, BLAST, QMEAN servers, SWISS-PROT, QMEAN servers, STRING and Rampage validation tool.

Introduction:-

Bioinformatics is the discipline of science in which biology, computer science, and information technology merge to form a single command. A web server for the analysis and comparison of 2D gels using bioinformatics tools has been developed, [1]. Functional analysis and elucidation of large-scale proteomics and gene expression data require effective use of bioinformatics tools and collective information resources coupled with expert-guided examination [2]. Bisphenol-A (BPA), one of many environmental endocrine disrupters, is widely used in polycarbonate plastics, food cans and dental sealants. It is generally believed that consumer exposure to BPA occurs primarily via food in contact with BPA-containing materials, such as polycarbonate baby bottles, table ware and food containers as well as food and beverage cans lined with epoxy resins. Differences in the estrogenic activity of bisphenol A and reference estrogens may be due to differences in recruiting by the liganded receptor of co-regulatory proteins. BPA is thought to bind to plasma proteins in rodents, monkeys and humans [3]. Because pharmacokinetics are altered by protein binding, the potential uptake of BPA into other tissues, including estrogen-target tissues, may be affected. In proteomics ground, combinations of analytical techniques are used to analyse the protein samples. The initial step in all proteomic studies involves the separation of a mixture of proteins. This can be conceded out using 2-D gel electrophoresis technique in which proteins are separated based on their individual molecular weight and charges. Two dimensional gel electrophoresis can retrieve information regarding thousands of different proteins from a crude protein sample. The spots obtained in 2-D gel electrophoresis are separated and subjected to mass spectrometric analysis using MALDI-TOF. MALDI-TOFMS/MS data converted into MGF (Mascot Generic Format). This data can be analysed by using MASCOT server. Mascot is widely used by research facilities around the world. Mascot uses a probabilistic scoring algorithm for protein identification that was adapted from the MOWSE algorithm.

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Mascot is a software search engine that uses mass spectrometry data to identify proteins from peptide sequence databases [4]. This sequence is aligned by using alignment tools, sequence alignment is to identify the functional and structural relationship between the sequences [5].The first step in comparative modelling is to distinguish all protein structures related to the target sequence, some of which will be used as templates. This step is greatly facilitated by databases of protein sequences and structures and software for scanning those databases. The target sequence can be searched against sequence databases, such as Protein Identification Resource (PIR), GENBANK , SWISS-PROT, or EMBO nucleotide sequences database , and/or structure databases such as the Brookhaven Protein Databank and SCOP . The most popular programs, including FASTA and BLAST to compare the target sequence with each sequence in a database Program MODELLER which implements all the stages in comparative modelling [6], can also automatically search for proteins with known three-dimensional structure that are related to a given sequence. QMEAN is a composite scoring function which is able to derive both entire structure and residue of protein error estimates on the basis of one single model [7,8].

Experimental:-

Tissue collection:-

The hippocampal regions of the brain were dissected and then stored at -80˚C until use. The tissue was homogenized on ice with a cold Tris/EDTA buffer and centrifuged at 10,000 g for 20 min at 48˚C. Supernatant was collected and processed for protein analysis. [9] About 0.5g of each liver was homogenized in 4.5 ml of phosphate buffered saline.The crude tissue was centrifuged at 8000 g for 30 min and the supernatant was collected and stored at 4˚C [10]

2D gel electrophoresis:-

The samples were loaded on to the IEF strips 3-10pH Linear, 18cm and kept for Iso-Electric Focusing. After IEF run, the strip was equilibrated in Equilibration Buffer and the second dimension was carried out on a 10% SDS-PAGE. The gels were Silver stained to observe the protein spots and were scanned using Epson Expression 11000XL Scanner

MS Analysis:-

Mass spectrometry is an important emerging method (Model voyager De-STR, applied Biosystems, Foster, CA, USA) for the characterization of proteins from isolated 2-D gel spots as this method is very sensitive. Spot was treated with acetonitrile for dehydration and trypsin for protein digestion. α-cyano-4-hydroxycinnamic acid in acetonitrile was used as matrix.

MASCOT Search:-

After MALDI-TOF MS/MS analysis expressed protein data is converted into MGF (MascotGeneric Format). These data can be analysed by using MASCOT server(http://www.matrixscience.com). Mascot has three main search modes: Peptide MassFingerprint, Sequence Query, and MS/MS Ion Search. MS/MS Ion Search is used to analyse data from tandem mass spectrometry experiments. The report was generated depending on specific options used for protein analysis. For each protein match, Mascot calculates an overall Protein Score. This number reflects the combined scores of all observed mass spectra that can be matched to amino acid sequences within that protein. A higher score indicates a more confident match. The number of protein matches at each scoring position is indicated by the height of the red bars, the non-significant area is shaded in green. Complete results are automatically sent to the registered E-mail.

Sequence alignment:-

The target sequence was searched with BLAST search against Protein Data Bank, which one has a high level of sequence identity with target protein selected as a template protein. Templates were determined by super imposition of the two structures and multiple sequence alignment was performed with CLUSTAL W (11) program to identify the set of conserved residues alignment.

Homology modeling:-

The sequences were analysed with the help of Phyre-2 (protein Homology/analogy Recognition Engine V 2.0) server for obtaining pdb file. The final 3-D structure obtained with the help of RasMol version 2.6 software programme.

Ramachandran plot analysis:-
Ramachandran plot displays the phi and psi backbone conformational angles for each residue in a protein. The phi angle is the angle of right-handed rotation around N-Cα bond and the psi angle is the angle of right-handed rotation around Cα-C bond. Phi and psi angles are also used in the classification of some secondary structure elements such as alpha helix and beta turns. In a Ramachandran plot, the core or allowed regions indicate preferred areas for psi/phi angle pairs for all residues in a protein. If the determination of protein structure is reliable, most pairs will be in the favoured regions of the plot, some pairs will be in the allowed region, and only a few will appear in 'disallowed' regions

QMEAN analysis for the quality resolution structure:-
The QMEAN scoring function estimates the global quality of the models on the basis of a linear combination of six structural descriptions, four of them are statistical potentials of mean force. The local geometry is analysed by a torsion angle potential over three consecutive amino acids. The distance –dependent interaction potentials based on Cβ atoms and all atoms, respectively are used to assess long-range interactions. A solvation potential describes the burial status of the residues. The analysis of these Z-scores of the individual terms can help identifying the geometrical features responsible for an observed large negative QMEAN Z-score. Models of low quality are expected to have strongly negative Z-scores for QMEAN but also for most of the contributing terms. Large negative values correspond to red regions in the colour gradient. Good structures are expected to have all sliders in the light red to blue region. The quality of resolution structure of differentially expressed proteins under control, bisphenol and bisphenol with probiotic treated samples represented in the following figures

Analysis of physico-chemical parameters of a sequence:-
Protparam is one among the protein analysis tool available on the ExPasy server. (http://www.expasy.org/tools/protparam.html).It is used for calculating various physiochemical parameters of a provided protein. The protein can be either is specified as a UniProtKB/Swiss-Prot accession number or ID or as sequences of amino acids.

String analysis of differentially expressed proteins:-
The database STRING is a precomputed global resource for the exploration and analysis of these associations. Since the three types of evidence differ conceptually, and the number of predicted interactions is very large, it is essential to be able to assess and compare the significance of individual predictions. Protein–protein interactions are not limited to direct physical binding. Proteins may also interact indirectly by sharing a substrate in a metabolic pathway, by regulating each other transcriptionally, or by participating in larger multi-protein assemblies. For information on genomes, genes, and encoded proteins, STRING relies on the annotated proteomes maintained bySWISS-PROT.

Results:-

Table 1:- List of differentially expressed proteins of hypothalamus tissue of *Rattus norvergicus* in response to BPA and BPA with probiotic treatement.

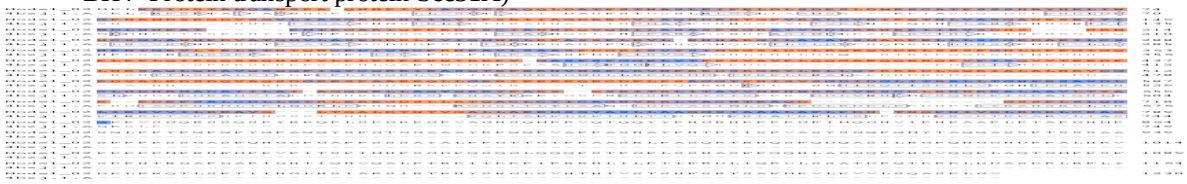
Spot no	Protein name	Molecular weight	Calculated pI	Number of amino acids
BH3	gamma-actin, partial [Musmusculus]	41018.9	5.56	268
BH4	Protein transport protein Sec31A	133569.2	6.30	1230
BH5	Atp5b protein [Musmusculus]	56666.8	5.24	533
BPH3	PREDICTED: polyadenylate-binding protein 4 isoform X3	69331.3	9.58	630
BPH4	V-type proton ATPase catalytic subunit A	68326.0	5.41	617

Table 2:- List of differentially expressed proteins in liver tissue of *Rattus novegicus* in response to BPA and BPA with probiotic treatement

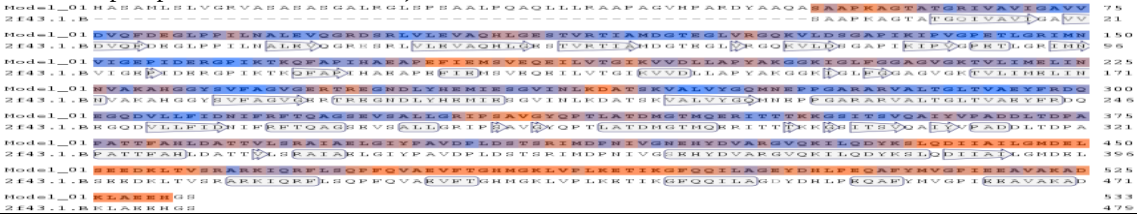
Spot no	Protein name	Molecular weight	Calculated pI	Number of Amino acids
BL1384	INSL3_MOUSE, Insulin-like 3	13585.8	9.25	122
BL1500	ATP synthase subunit beta, mitochondrial precursor [Musmusculus]	56300.4	5.19	529
BL1549	Laminin subunit alpha-5 precursor	404053.6	6.28	3718
BPL119	Nucleolar protein 14	98769.4	7.34	860
BPL128	Nuclear protein MDM1	75673.7	9.33	673

a) gamma-actin, partial [Musmusculus] target protein (3ub5 selected as a reference structure for modeling of BH3- gamma-actin, partial [Musmusculus])

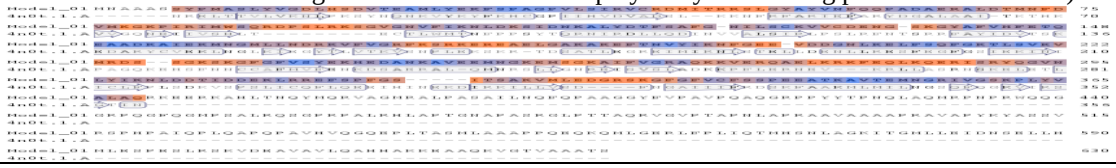
b) Protein transport protein Sec31A target protein (4bzj selected as a reference structure for modeling of BH4- Protein transport protein Sec31A)



c) Atp5b protein [Musmusculus] target protein(2f43 selected as a reference structure for modeling of BH5 Atp5b protein [Musmusculus])



d) PREDICTED: polyadenylate-binding protein 4 isoform X3 target protein(4n0t selected as a reference structure for modeling of BPH3-PREDICTED: polyadenylate-binding protein 4 isoform X3).



e) V-type proton ATPase catalytic subunit A target protein(3j9tselected as a reference structure for modeling of BPH4- V-type proton ATPase catalytic subunit A)

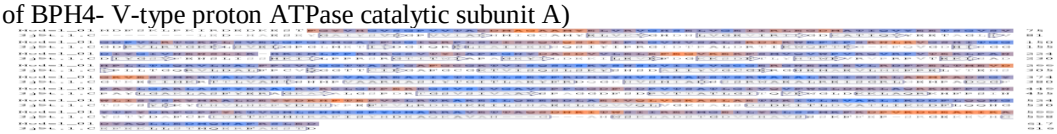
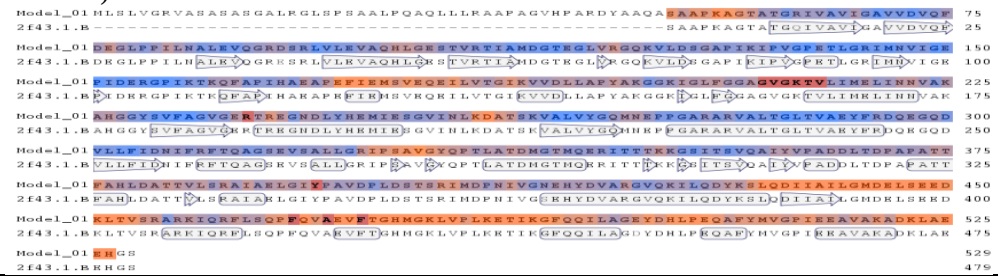


Fig 1:- Sequence alignment of differentially expressed proteins In hypothalamus induced by Bisphenol, Bisphenol and probiotic treated proteins.

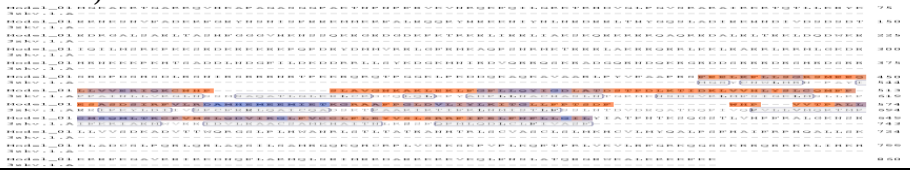
f) Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit target protein(2f43 selected as a reference structure for modeling of BL 1500- Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit).



g) Laminin subunit alpha-5 precursor target protein(2y38selected as a reference structure for modeling of BL1549- Laminin subunit alpha-5 precursor).

h) INSL3_MOUSE, Insulin-like 3 target protein(2kqp selected as a reference structure for modeling of BL1384-INSL3_MOUSE, Insulin-like 3).

i) Nucleolar protein 14 target protein(3zkv selected as a reference structure for modeling of BPL 119- Nucleolar protein 14).

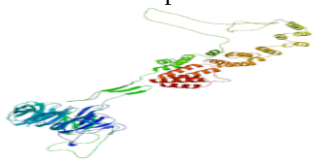


j) Nuclear protein MDM1 target protein (1wpbselected as a reference structure for modeling of BPL 128- Nuclear protein MDM1).

Fig 2:- Sequence alignment of differentially expressed proteins In liver induced by bisphenol , Bisphenol and probiotic treated proteins

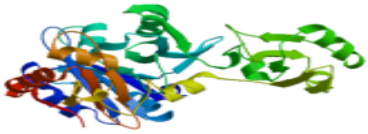
a) 3Dimensional modeled structure of BH3 gamma-actin partial [Musmusculus] protein

b) 3 Dimensional modeled structure of Protein transport protein Sec31A protein



c) 3 Dimensional modeled structure of BH5-Atp5b protein [musmusculus]

d) 3 Dimensional modeled structure of BPH3-PREDICTED: polyadenylate-binding protein 4 isoform X3 protein



e) 3 Dimensional modeled structure of BPH4- V-type proton ATPase catalytic subunit A protein

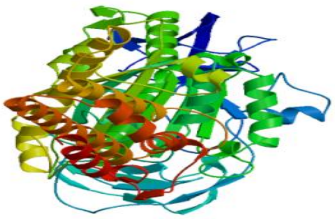
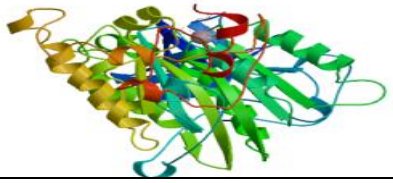


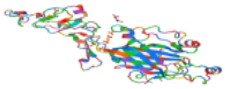
Fig 3:- Homology modeling of differentially expressed proteins of Hypothalamus induced by Bisphenol A and treatement with BPA+probiotic bacteria

f) 3Dimensional structure of BL 1500-Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit protein

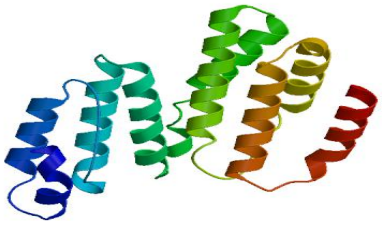


g) 3Dimensional structure of BL1384-INSL3_MOUSE, Insulin-like 3 protein

h) 3Dimensional structure of BL1549-Laminin subunit alpha-5 precursor protein



i) 3 Dimensional structure of BPL 119-Nucleolar protein 14 protein



j) 3Dimensional structure of BPL 128-Nuclear protein MDM1 protein

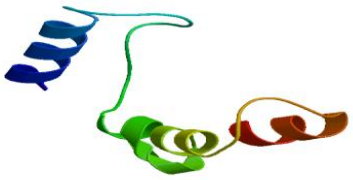
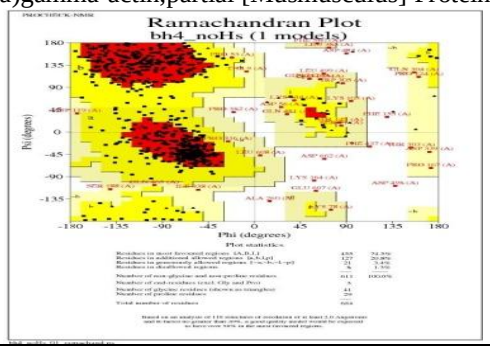


Fig 4:- Homology modeling of differentially expressed proteins of Liver induced by BPA and treatemnt with BPA + probiotc bacteria

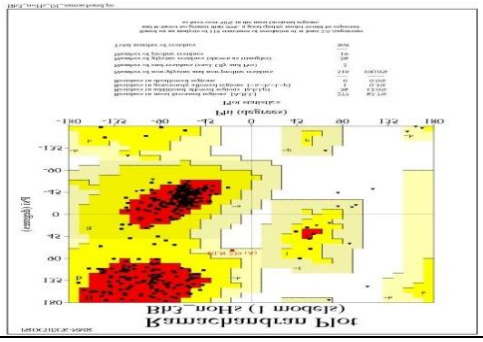
Table 3:- Validation of Hypothalamus tissue protein sample by Ramachandran's plot analysis

S.No	No. of residues in most favoured regions	No.of residues in additional allowed regions	No.of residues in disallowed regions	No.of glycine and proline residues
1.	gamma-actin,partial [Musmusculus]	277(87.7%)	38(12.0%)	28 (Gly),19(pro)
2.	Protein transport protein Sec31A	455(74.5%)	127(20.8%)	41(Gly),29(pro)
3.	Atp5b protein [Musmusculus]	363(91.9%)	30(7.6%)	45 (Gly), 24 (pro)
4.	PREDICTED: polyadenylate-binding protein 4 isoform X3	284(87.1%)	31(9.6%)	28 (Gly), 9(pro)
5.	V-type proton ATPase catalytic subunit A	458(83.4%)	50(9.7%)	50 (Gly), 30(pro)

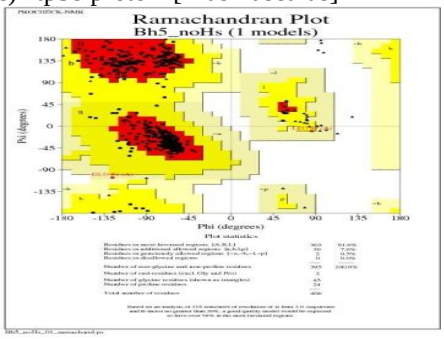
a)gamma-actin,partial [Musmusculus] Protein



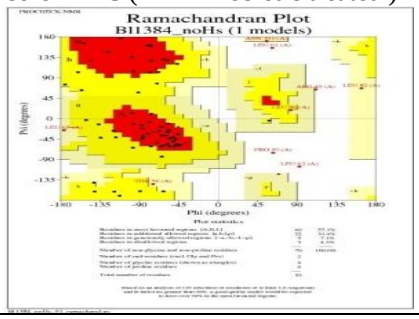
b)Protein transport protein Sec31A



c)Atp5b protein [Musmusculus]



d) PREDICTED: polyadenylate-binding protein 4 isoform X3 (BPA+Probiotic treated)



e) V-type proton ATPase catalytic subunit A(BPA+Probiotic treated).

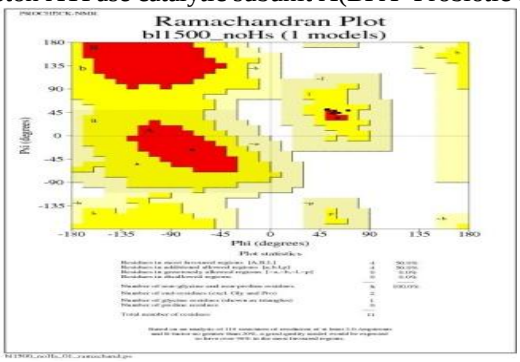
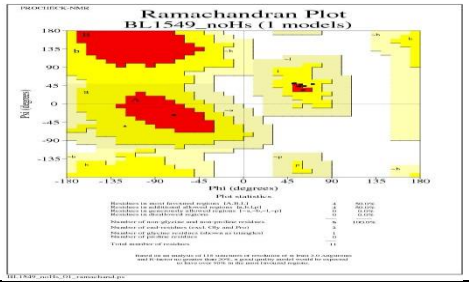


Fig 5:- Illustration of Ramachandran's plot analysis of proteins expressed in Hypothalamus response to BPA stress and BPA+Probiotic treatement

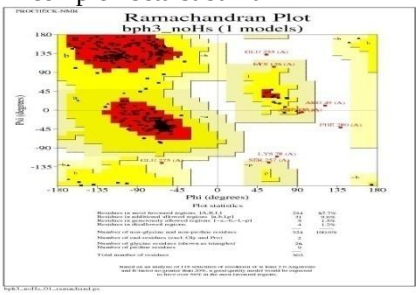
Table 4: Validation of Liver tissue proteins by Ramachandran's plot analysis

S.No	No. of residues in most favoured regions	No.of residues in additional allowed regions	No.of residues in disallowed regions	No.of glycine and proline residues
6.	INSL3_MOUSE, Insulin-like 3	40(57.1%)	22(31.4%)	5 (Gly), 6(pro)
7.	Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit	4(50.0%)	4(50.0%)	1 (Gly), 0 (pro)
8.	Laminin subunit alpha-5 precursor	44(50.0%)	4(50.0%)	1 (Gly), 0 (pro)
9.	Nucleolar protein 14	103(89.6%)	11(9.6%)	5 (Gly), 7(pro)
10.	Nuclear protein MDM1	4(50.0%)	4(50.0%)	1(Gly), 0(pro)

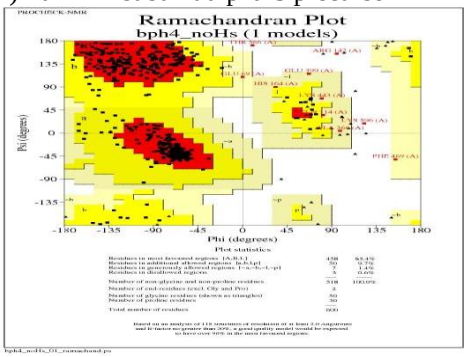
f) INSL3_MOUSE, Insulin-like 3



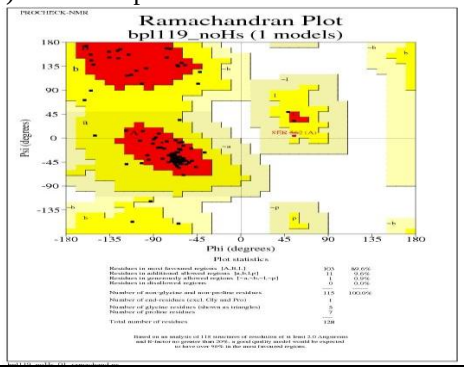
g) Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit



h) Laminin subunit alpha-5 precursor



i) Nucleolar protein 14



j)128-Nuclear protein MDM1

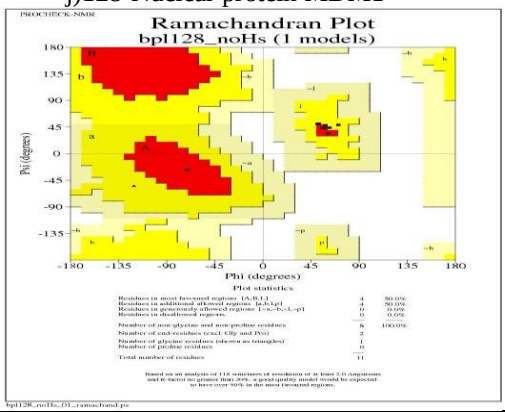
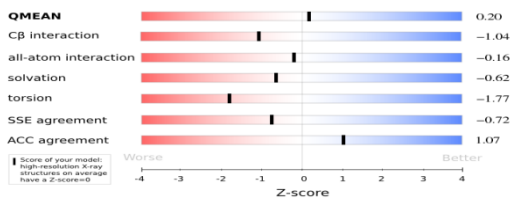


Fig 6:- Ramachandran's plot analysis of proteins expressed in Liver response to BPA stress and BPA+Probiotic treatement.

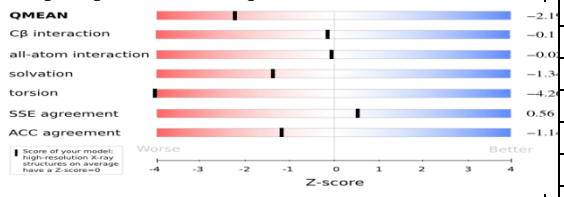
a)Quality of resolution structure of -gamma-actin, partial [Musmusculus] protein



QMEAN scoring function of gamma-actin, partia[Musmusculus]

Protein model name	BH3
Zscore_QMEAN	0.202664453420632
Zscore_Cbeta	1.03958792614991
Zscore_all_atom	0.161708866011722
Zscore_solvation	0.622112948131572
Zscore_torsion	1.76687838179354
Zscore_SSE_agree	0.721091515598544
Zscore_ACC_agree	1.06896443585087

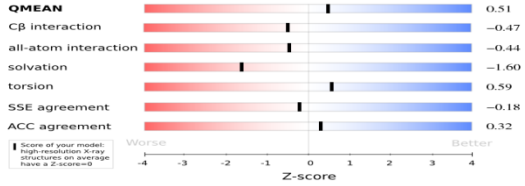
b) Quality of resolution structure of Protein transport protein Sec31 protein



QMEAN scoring function of Protein transport protein Sec31A

Protein model name	BH4
Zscore_QMEAN	2.19243199536967
Zscore_Cbeta	0.109382670524617
Zscore_all_atom	0.0201402306006502
Zscore_solvation	1.34093329731162
Zscore_torsion	4.25948482492861
Zscore_SSE_agree	0.560296498026475
Zscore_ACC_agree	1.14302010241546

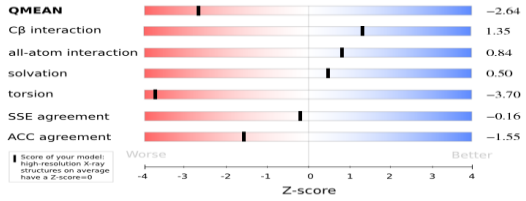
c) Quality of resolution structure of Atp5b protein [Musmusculus] protein



QMEAN scoring function of Atp5b protein [Musmusculus]

Protein model name	BH5
Zscore_QMEAN	0.508027357752407
Zscore_Cbeta	0.469305344908218
Zscore_all_atom	0.439180122160005
Zscore_solvation	1.59520535256758
Zscore_torsion	0.592791846376968
Zscore_SSE_agree	0.180361826190693
Zscore_ACC_agree	0.323508715703452

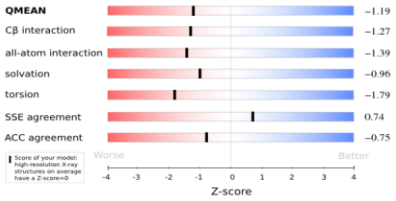
d) Quality of resolution structure of PREDICTED: polyadenylate-binding protein 4 isoform X3 protein



QMEAN scoring function of -PREDICTED: polyadenylate-binding protein 4 isoform X3

Protein model name	BPH3
Zscore_QMEAN	2.64365830791129
Zscore_Cbeta	1.35236987594616
Zscore_all_atom	0.841938004887182
Zscore_solvation	0.500248807406608
Zscore_torsion	3.70379067315507
Zscore_SSE_agree	0.161966738028873
Zscore_ACC_agree	1.54929393635679

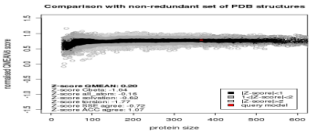
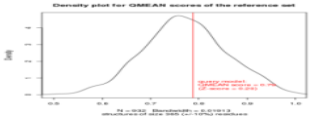
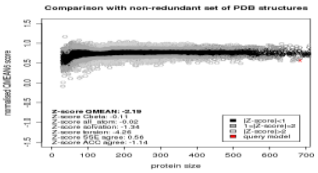
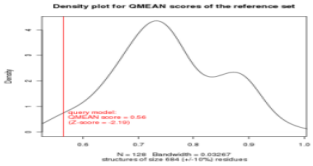
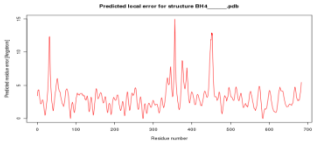
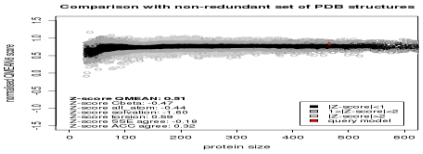
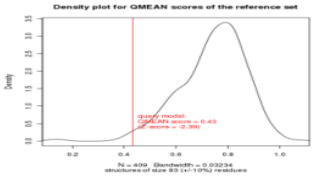
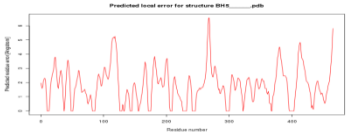
e)Quality of resolution structure of V-type proton ATPase catalytic subunit A protein



QMEAN scoring function of V-type proton ATPase catalytic subunit A

Protein model name	BPH4
Zscore_QMEAN	1.18765547487996
Zscore_Cbeta	1.26927752065401
Zscore_all_atom	1.39075172759304
Zscore_solvation	0.963680705267424
Zscore_torsion	1.79409653465914
Zscore_SSE_agree	0.744410029116678
Zscore_ACC_agree	0.752399634822914

Fig 7:- QMEAN analysis for the quality resolution structure of Hypothalamus proteins induced with BPA and BPA+Probiotic treatement.

a) Qualityestimation of BH3-gamma-actin,partial [Musmusculus] 	Density plot for BH3- gamma-actin, partial [Musmusculus] 	Predicted local error for BH3-gamma-actin, partial [Musmusculus] 
b) Quality estimation of BH4-Protein transport protein Sec31A 	Density plot forBH4- Protein transport protein Sec31A 	Predicted local error for BH4-Protein transport protein Sec31A 
c) Quality estimation of BH5-Atp5bprotein[Musmusculus] 	Density plot forBL1384-INSL3_MOUSE, Insulin-like 3 	Predicted local error for BH5- Atp5b protein [Musmusculus] 

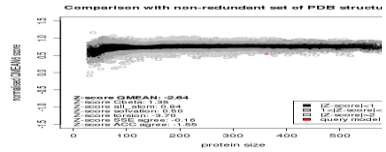
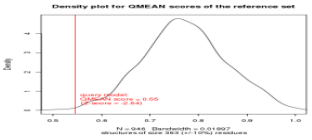
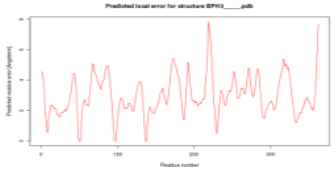
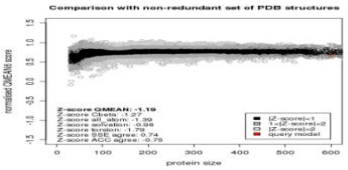
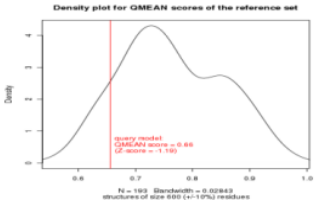
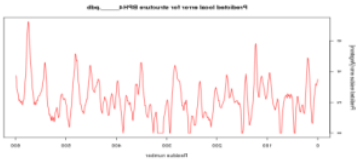
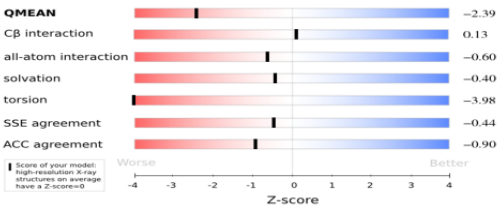
d) Quality estimation of BPH3-PREDICTED: polyadenylate-binding protein4isoformX	Density plot forBPH3-PREDICTED: polyadenylate-binding protein 4 isoform X3	Predicted local error for BPH3 -PREDICTED: polyadenylate-binding protein 4 isoform X3
		
e) Quality estimation of BPH4- V-type proton ATPase catalytic subuni	Density plot forBPH4- V-type proton ATPase catalytic subunit A	Predicted local error for BPH4- V-type proton ATPase catalytic subunit A
		

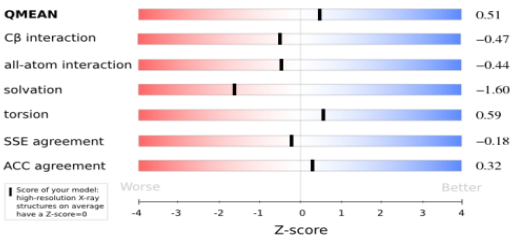
Fig 8:- Qmean Analysis,Density Plot Analysis ,Predicted Local Error for estimating the quality of differentially expressed proteins structure of Hypothalamus tissue induced with BPA and BPA+Probiotic treatement

f)Quality of resolution structure of 1384-INSL3_MOUSE, Insulin-like 3 protein



Protein model name	BL1384
Zscore_QMEAN	2.38964453671237
Zscore_Cbeta	0.127878246354725
Zscore_all_atom	0.597864567752103
Zscore_solvation	0.4017765313089
Zscore_torsion	3.97626544639136
Zscore_SSE_agree	0.436430019683796
Zscore_ACC_agree	0.895632370321055

g)Quality of resolution structure of 1500-Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit protein

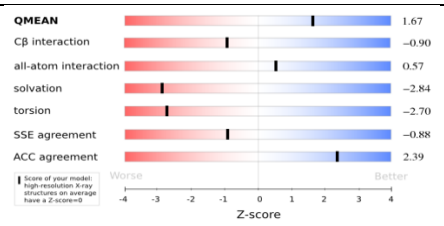


Protein model name	BL1500
Zscore_QMEAN	0.508027357752407
Zscore_Cbeta	0.469305344908218
Zscore_all_atom	0.439180122160005
Zscore_solvation	1.59520535256758
Zscore_torsion	0.592791846376968
ZscoreSSEagree	0.180361826190693
ZscoreACCagree	0.323508715703452

h)Quality of resolution structure of 1549- Laminin subunit alpha-5 precursor protein

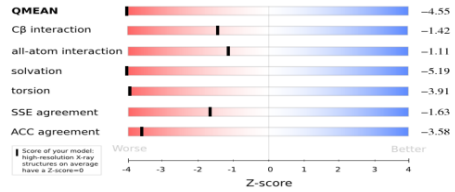
QMEAN scoring function of BL1549- Laminin subunit alpha-5 precursor

Protein model name	BL1549
Zscore_QMEAN	1.66677810992402
Zscore_Cbeta	0.895601151162753
Zscore_all_atom	0.567516734841358



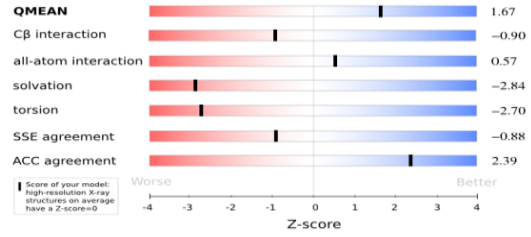
Zscore_solvation	2.83634683268503
Zscore_torsion	2.70025070918719
Zscore_SSE_agree	0.88335614306389
Zscore_ACC_agree	2.39413473586966

i)Quality of resolution structure of 119- Nucleolar protein 14 protein



Protein model name	BPL119
Zscore_QMEAN	4.55307497044615
Zscore_Cbeta	1.41575152531102
Zscore_all_atom	1.10860166383448
Zscore_solvation	5.1865902138161
Zscore_torsion	3.90984298295946
Zscore_SSE_agree	1.62704227512068
Zscore_ACC_agree	3.57947996825142

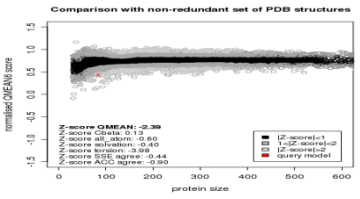
j)Quality of resolution structure of 128-Nuclear protein MDM1 protein



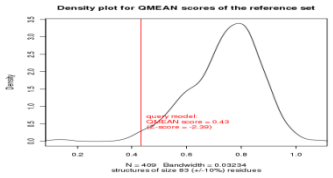
Protein model name	BPL128
Zscore_QMEAN	1.66677810992402
Zscore_Cbeta	0.895601151162753
Zscore_all_atom	0.567516734841358
Zscore_solvation	2.83634683268503
Zscore_torsion	2.70025070918719
Zscore_SSE_agree	0.88335614306389
Zscore_ACC_agree	2.39413473586966

Fig 9:- QMEAN analysis for the quality resolution structure of Liver proteins induced with BPA and BPA+Probiotic treatement.

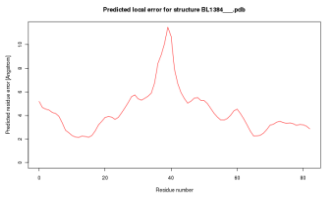
a)Quality estimation of BL1384-INSL3_MOUSE, Insulin-like



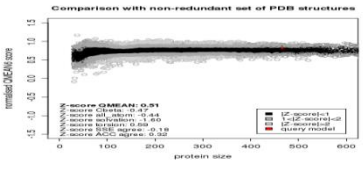
Density plot forBL1384-INSL3_MOUSE, Insulin-like 3



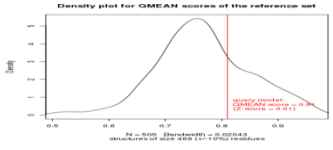
Predicted local error for BL1384-INSL3_MOUSE, Insulin-like 3



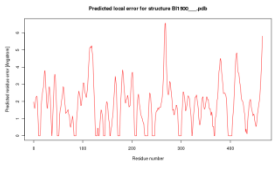
Quality estimation of BL 1500-Mitochondrial ATP synthase, H+ transporting F1 complexbeta subunit



Density plot forBL 1500-Mitochondrial ATP synthase, H+ transporting F1 complex beta subuniT



Predicted local error for BL 1500-Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit



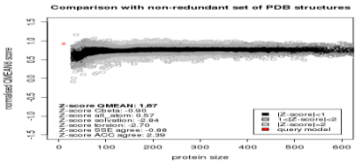
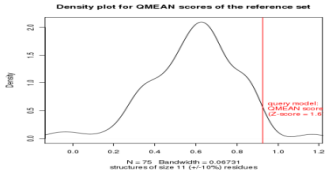
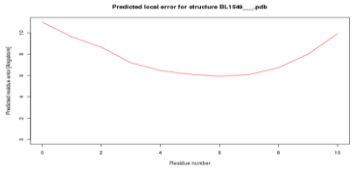
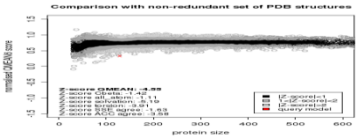
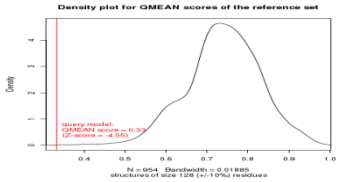
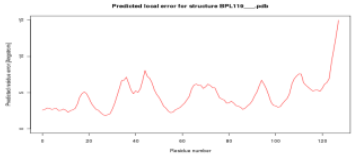
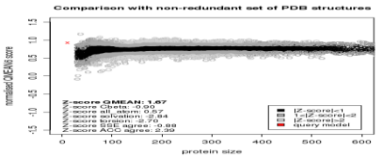
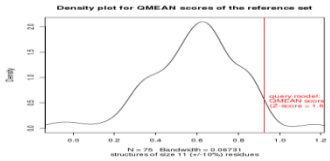
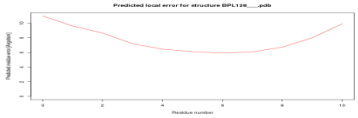
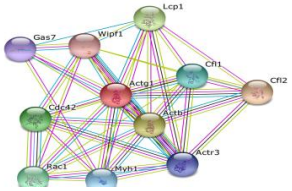
Quality estimation of BL1549- Laminin subunit alpha-5 precursor 	Density plot forBL1549- Laminin subunit alpha-5 precursor 	Predicted local error for BL1549-Laminin subunit alpha-5 precursor 
Quality estimation of BPL 119-Nucleolar protein 14 	Density plot forBPL 119- Nucleolar protein 14 	Predicted local error for BPL 119-Nucleolar protein 14 
Quality estimation of BPL 128-Nuclear protein MDM1 	Density plot forBPL 128-Nuclear protein MDM1 	Predicted local error for BPL 128-Nuclear protein MDM1 

Fig 10:- Qmean Analysis,Density Plot Analysis ,Predicted Local Error for estimating the quality of differentially expressed proteins structure of Liver tissue induced with BPA and BPA+Probiotic treatement

Prot para analysis of gamma-actin, partial [Musmusculus] protein

Formula of the protein	C1822H2860N484O548S22
Total No.of atoms	5736
Extinction coefficient	44725
Molecular weight	41018.9 Daltons
Estimated half-life	5.5
Instability index	36.18 (Stable Protein)
Aliphatic index	82.17
GRAVY (Grand average of hydropathicity)	-0.201

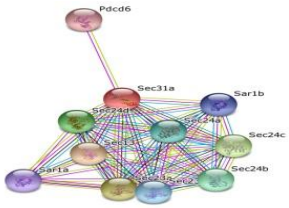
String structure **of** gamma-actin, partial [Musmusculus



Prot param analysis of Protein transport protein Sec31A

Formula of the protein	C5910H9310N1638O1811S40
Total No.of atoms	18709
Extinction coefficient	129355
Molecular weight	133569.2 Daltons
Estimated half-life	30 hours
Instability index	54.48 (Unstable Protein)
Aliphatic index	79.21
GRAVY(Grand average	-0.376

String structure of Protein transport protein Sec31A

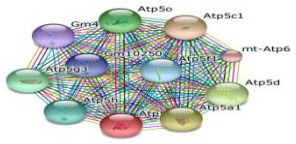


of hydropathicity)	

Prot param analysis of Atp5b protein [Musmusculus]

Formula of the protein	C2517H4062N688O768S13
Total No.of atoms	4062
Extinction coefficient	19370
Molecular weight	56666.8 Daltons
Estimated half-life	3.5 hours
Instability index	34.99 (Stable Protein)
Aliphatic index	99.96
GRAVY(Grand average of hydropathicity)	0.032

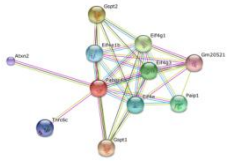
String structure of Atp5b protein [Musmusculus]



Prot param analysis of PREDICTED: polyadenylate-binding protein 4 isoform X3

Formula of the protein	C3057H4876N882O905S27
Total No.of atoms	9747
Extinction coefficient	38070
Molecular weight	69331.3 Daltons
Estimated half-life	30 hours
Instability index	43.67 (Unstable Protein)
Aliphatic index	72.40
GRAVY(Grand average of hydropathicity)	-0.457

String structure of PREDICTED: polyadenylate-binding protein 4 isoform X3



Prot param analysis of V-type proton ATPase catalytic subunit A

Formula of the protein	C3040H4796N810O921S28
Total No.of atoms	9595
Extinction coefficient	70625
Molecular weight	68326.0 Daltons
Estimated half-life	30 hours
Instability index	35.10 (Stable Protein)
Aliphatic index	85.93
GRAVY(Grand average of hydropathicity)	-0.196

String structure of V-type proton ATPase catalytic subunit A

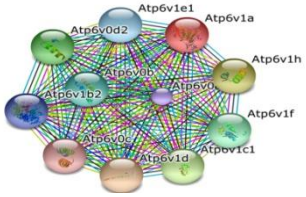
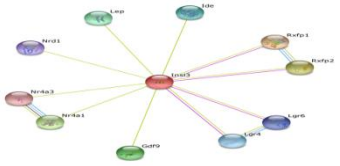


Fig 11:- Protparam and String analysis of expressed proteins in Hypothalamus tissue response to BPA and BPA+Probiotic treatement.

Protparam analysis of INSL3_MOUSE, Insulin-like 3

Formula of the protein	C587H970N190O165S8
Total No.of atoms	1920
Extinction coefficient	11375
Molecular weight	13585.8 Daltons
Estimated half-life	30 HOURS
Instability index	65.67 (Unstable Protein)
Aliphatic index	97.62
GRAVY(Grand average of hydropathicity)	-0.282

String structure of INSL3_MOUSE, Insulin-like 3

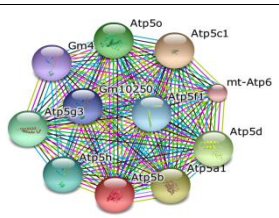


Protparam analysis of Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit

Formula of the protein	C2502H4040N682O763S13

String structure of Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit

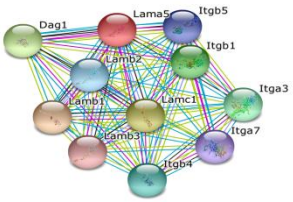
Total No.of atoms	8000
Extinction coefficient	19370
Molecular weight	56300.4 Daltons
Estimated half-life	30 hours
Instability index	35.18 (stable protein)
Aliphatic index	100.34
GRAVY(Grand average of hydropathicity)	0.033



Protparam analysis of Laminin subunit alpha-5 precursor

Formula of the protein	C17541H27400N5124O5339S267
Total No.of atoms	55671
Extinction coefficient	345030
Molecular weight	404053.6 Daltons
Estimated half-life	30 hours
Instability index	47.78 (Unstable Protein)
Aliphatic index	72.87
GRAVY(Grand average of hydropathicity)	-0.289

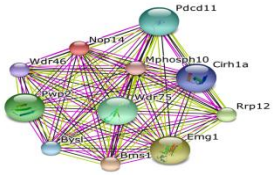
String structure of Laminin subunit alpha-5 precursor



Prot param analysis of Nucleolar protein 14

Formula of the protein	C4319H6963N1243O1334S35
Total No.of atoms	13894
Extinction coefficient	49110
Molecular weight	98769.4 Daltons
Estimated half-life	30 hours
Instability index	54.74 (Unstable Protein)
Aliphatic index	73.63
GRAVY(Grand average of hydropathicity)	-0.843

String structure of Nucleolar protein 14



Protparam analysis of Nuclear protein MDM1

Formula of the protein	C3285H5255N991O1035S15
Total No.of atoms	10581
Extinction coefficient	70735
Molecular weight	75673.4 Daltons
Estimated half-life	30 hours
Instability index	50.66 (Unstable Protein)
Aliphatic index	61.03
GRAVY(Grand average of hydropathicity)	-0.990

String structure of Nuclear protein MDM1

Fig 12:- Prot param and string analysis of expressed proteins in Liver tissue response to BPA and BPA+probiotic treatement

Discussion:-

In the present study, we have identified the structures and complete protein information regarding 3D structures, atomic configurations, no.of amino acid residues in ramachandrans plot analysis of BPA stressed liver proteins and BPA and probiotic treated Hypothalamus tissue proteins. We were interested in BPA induced proteins in liver as they were over expressed and this over expression in normal cellular metabolism may lead to the diseases.

INSL3_MOUSE,insulin-like3 protein:-

This protein is a member of the insulin like hormone or protein super family and was first recognized by cloning projects using testicular tissue, hence its original name of Leydig insulin-like peptide [12,13] and also expressed in

ovarian theca and luteal cells in females. Although recognized for some time, its function was unknown until 1999 when two groups investigating mice mutants for INSL3 found bilateral cryptorchidism and developmental abnormalities of the gubernaculums [14,15] in males. It is the Newest hormone or protein demonstrated to be involved in abnormalities like:

Testicular descent or cryptorchidism and Gubernacular swelling:-
INSL3/Leydig insulin-like peptide acts as a ligand and activates the LGR8 receptor (G-protein coupled receptor) present on testes cells, important in testis descent. This LGR8 receptor activation leads to Signalling process and INSL3 peptide continous signalling or mutation leads to a condition called cryptorchidism in which testicle that doesn't move into its proper position in the bag of skin hanging below the penis (scrotum). In humans, circulating INSL3 increases through puberty, to reach a maximum in early adulthood, and subsequently appears to decline to significantly lower plasma levels in aging men [16, 17]. Recently, it has been suggested [18] that androgens and phthalates at high concentration may modulate Insl3 gene expression in cultured Leydig cells. Caudal enlargement of the gubernaculum during relative transabdominal movement of the testis is known as the " gubernacular swelling reaction" or "gubernacular outgrowth" and is caused by cell division and an increase in glycosaminoglycans and hyaluronic acid (19). The hydrophilic nature of hyaluronic acid makes the end of the gubernaculum bulky and gelatinous. In females over expression of INSL3 induces ovary descent.

Mitochondrial ATP synthase, H+ transporting F1 complex beta subunit protein:-
Mitochondrial ATP5B expression in the liver has been shown to be controlled at the post-transcriptional level (and controlling process was found to be induced by miR-127-5p) .MiR-127-5p 3'UTR of β-F1-ATPase which shows much expression in fetal liver targets mRNA (β-mRNA) and miR-127-5p inhibits β-F1-ATPase mRNA translation in humans [20]. MiR-127-5p has an important role in regulating the activity of mitochondrial bioenergetics in oncogenesis[21]. ATP5B was found to be up regulated in breast cancer in tissues in a significant manner. Control of translational efficiency of beta-F1- ATPase mRNA depends on the regulation of a protein that binds the 3' untranslated region of the m RNA. miR-127-5p inhibits β-F1-ATPase mRNA translation in humans.

Counter effect on ATP synthase:-
The agonist of ATP synthase MAb3D5AB1 recognises catalytic β-subunit of ATP synthase and inhibits the activity of F1domain. Mab3d5ab1 shows angiostatin-like properties and can be useful in the chemotheraaphy... This protein can even play an important role as a target protein in the treatment of cancers. Using ATP synthase inhibitor aurovertin B, in breast cancer cells MCF-7, the effect of ATP5B protein in tumor progression was found to be reduced. [22].

Laminin subunit alpha-5 precursor protein:-
This protein contains five, N-terminus, extracellular immunoglobulin domains, a single transmembrane domain, and a short, C-terminal cytoplasmic tail and may play a role in epithelial cell cancer and in vaso-occlusion of red blood cells in sickle cell disease. More recent data indicate a direct participation of the vascular endothelium, of multiple and complex cellular interactions, and of a global inflammation-mediated cell activation, in the initiation and propagation of the vaso-occlusive process with two consecutive steps. The first step involves adhesion of the stress reticulocytes[23] and activated polymorphonuclear neutrophils8 , (iii) signalling pathways in the red blood (the signalling pathways in the red blood cell, makes the cell susceptible to be modulated by stress, hypoxia, and by the inflammatory response and to influence the activation status of adhesion receptors and of ion transporters implicated in SS-RBC dehydration and finally of a syndrome of complex endothelial dysfunction involving abnormalities of the metabolism of nitric oxide (NO))was brought into light to the endothelium of post-capillary veinules, slowing down the blood flow and thereby inducing and propagating sickling of mature SS-RBCs that are maintained for a longer time in a hypoxic environment and activates polymorphonuclear neutrophils second step involves the entrapment of irreversible sickle cells and to the complete occlusion of the micro-vessels[24-31]

Conclusion:-

In our study, we concluded that BPA stressed proteins in liver tissue are involved in certain metabolic disorders like cryptorchidism , Gubernacular swelling ,oncogenesis, epithelial cell cancers and Vaso-occlusive process where BPA and probiotic treatement in liver shown production of Nuclear protein MDM1 protein which is a microtubule-binding protein that negatively regulates centriole duplication which binds and stabilizes microtubules in controlling cell duplication process in testis. This negative regulation seems to be to control the cryptochidism caused by over expressed proteins by BPA in liver.

Conflict Of Interest:-
We declare that we have no conflict of interest.

Acknowledgement:-

The authors are thankful to UGC, New Delhi for providing financial assistance (UGC-MRP).

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