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Molecular Docking and *In Silico* Pharmacokinetics of Biochanin-A as Novel Antidepressant

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Abstract: Present study was an attempt to investigate the bioactive compound biochanin-A for its antidepressant potential using molecular docking studies. The molecular docking studies were done using the commercial docking software MCULE, 1- click docking. Docking study revealed that biochanin-A interacted well with the active sites of different targets of monoamine, neuroinflammatory, oxidative stress and some selective marker proteins. Among mono amine target proteins selected, biochanin-A showed good binding affinity with monoamine oxidase MAO-A (PDB ID 2BXR) with a docking score of -9.0 kcal/mol. Similarly it showed best interaction with active sites of Cyclo-oxygenase (COX-2) (PDB ID 4COX) with a docking score of -9.2 kcal/mol among inflammatory targets and Nitric Oxide synthase (NOS2-PDB ID 3HR4) with a docking score of -9.2 kcal/mol among oxidative stress related targets. It showed good interaction with Heat Shock Protein (HSP70-PDB ID3JXU), with a docking score of -9.2 kcal/mol. Among all targets biochanin-A showed highest docking score compared to standard drugs like imipramine, fluoxetine, diclofenac and ascorbic acid. *In silico* ADME evaluation of biochanin-A showed good percentage of gastrointestinal absorption and free of major toxicities.

Keywords: Biochanin-A, *In silico,* Docking, Anti-depressant, monoamine oxidase, cyclo-oxygenase, Matrix Metallo Peptidase, Nuclear Factor Kappa Light Chain Enhancer of Activated B Cells, Interleukin-1 Receptor- Associated Kinase 4, Nitric Oxide Synthase, Heat Shock Protein, Vascular Endothelial Growth Factor Receptor-2, Peroxisome Proliferator Activated Receptor- γ

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Introduction

Phytoestrogens are also referred as "dietary estrogens" and naturally occurring compounds having structural similarity with mammalian estrogen (17-β-estradiol), act as agonist or

antagonist i.e. estrogens or antiestrogens (Gruber *et al*., 2002; Rietjens *et al*., 2017). Phytoestrogens are considered as potent molecules aganist cancer, diabetes, osteoporosis, obesity and cardiovascular diseases (Miadokova, 2009). The Biochanin A or 5, 7-Dihydroxy-4'-methoxy isoflavone 5, or 7-dihydroxy-3-(4- methoxyphenyl) chromen-4-one is an O-methylated isoflavone. Phytoestrogens exhibit various pharmacological activities including neuroprotective, anti-cancer, anti-oxidant, anti-inflammatory, anti-allergic, anti-hyperglycemic and anti-hyperlipidemic effects (Seo *et al*., 2011; Harini *et al*., 2012; Chung *et al*., 2013; Wang *et al*., 2015; Sadri *et al*., 2017; Takahashi *et al*., 2017; Xue *et al*., 2017). Biochanin-A possess potent antioxidant, anti-inflammatory, neuroprotective properties and is more concentrated in the CNS (Sarfraz et al., 2020), it is selected as a novel ligand for the depression. Till to date its antidepressant potential was not explored hence, the present study was designed to evaluate the antidepressant potential of biochanin-A using molecular docking studies.

Major depressive disorders is discussed traditionally as a neurochemical disorder (Kendel *et al*., 2013). For decades depression is connected especially to interruption in serotonergic and noradrenergic neurotransmitters (Femina *et al*., 2001). Stressful events are the precipitating roots for the onset of depression (Marijeaan *et al*., 2009). Dysregulation of neurotransmitter levels result in the systemic effect with hyperactivation of hypothalamic pituitary adrenal axis (HPA) and results in hypercortisolemia creating a wide array of organ and immune changes (Wayne *et al*., 2008). The link of inflammation in depression is confirmed by the recent meta analysis, where elevated levels of C- Reactive protein (CRP), interleukin -1 (IL-1) and Interleukin-6 (IL-6) were reported in depression in clinical and community samples. Levels of Tumour Necrosis Factor (TNF-α) and IL-6 and blood levels of soluble Interluekin-2 receptors were higher in depressed patients. Depression is also characterized by a Th-1- like-cell- mediate response, with increased production of interferon-γ (IFN- γ) levels. Activation of NF-kB through Toll-like receptors (TLR) during immune challenge results in an inflammatory response through the release of the proinflammatory cytokines TNF- α, IL-1 and IL-6. These cytokines, enter the brain via leaky regions in the blood–brain barrier by active transport molecules and afferent nerve fibers (e.g. sensory vagus), which pass on information through the nucleus tractus solitarius (Maier and Watkins, 1998). In brain, cytokine signal pathways correlating the depression, alters metabolism of relevant neurotransmitters such as serotonin (5HT) and dopamine (DA) (Dunn *et al*., 1999; Gao *et al*., 2002), activates CRH in the paraventricular nucleus (PVN) and production (or) release of ACTH and glucocorticoids (cortisol) (Besedovsky and Del Rey, 1996; Silverman *et al*., 2005). Cytokine signal may disturb synaptic plasticity by modifications in relevant growth factors (e.g. BDNF-- brain-derived neurotrophic factor) (Lu *et al*., 2004) in depression. Environmental stressors further activate inflammatory signaling (NF-kB) through increased production of proinflammatory sympathetic nervous system responses [release of norepinephrine (NE), which binds to α (αAR) and β (βAR) adrenoceptors]. Stressors withdraw inhibitory motor vagal input [release of acetylcholine (ACh), which binds to the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR)] (Bierhaus *et al*., 2003; Pavlov and Tracey, 2005). Activation of the mitogen activated protein kinase (MAPK) pathways, including p38 and Jun amino-terminal kinase (JNK), inhibit the function of glucocorticoid receptors (GR), thereby releasing NF-kB from negative regulation by glucocorticoids (Wang *et al*., 2004; McKay and Cidlowski, 2004).

Bright *et al*. (2008) reported that PPAR agonist regulate inflammatory cytokines in CNS diseases by decreasing the TNF-α mRNA expression in antigenic-specific T cell *in vitro*. Activation of PPAR-γ in T lymphocytes decreased IL-2 production and proliferation. CNS injury resulted in rapid induction of microglia, macrophages, and astrocytes that secret IL-1, TNF-α, iNOS, PGs and COX-2 and PPAR-γ agonist inhibits them in dose

dependent manner. They also suggested and evidenced the role of PPAR-γ in regulation of IL-12 family cytokines, NF-$_k$B, and JAK-STAT signaling pathways in CNS diseases (Alzheimer's and multiple sclerosis) stating that PPAR-γ agonist regulates neuroinflammation in CNS diseases. Neuroinflammation results from the activation of the NF-$_k$B pathway which is activated by induction of CD40/TLR leading to expression of IL-12 family cytokines by antigen presenting cells (APCs), which in turn signal through Jak-stat pathway in T cells resulting in CNS disease. PPAR agonist prevents the CNS disease by modulating the signal transcription processes in APCs (Bright *et al*., 2008). Studies confirmed that NSAIDS, omega-3 fatty acids, minocyclines, statins and modafinil have significant antidepressant effects. But how this neuroinflammation interacts with the known neurobiological mechanisms of depression remains unclear (Troubat *et al*., 2020). Neuroinflammation is key factor that interact with neurobiological correlates of major depressive disorder and biochanin-A is a PPAR-γ agonist that may regulate the neuroinflammation and in turn depression.

In-silico methods are of great importance in Target identification and prediction of Novel drugs (Maithri *et al*., 2016). In the present study biochanin-A was docked to various monoamine, neuroinflammatory, oxidative stress and some selective markers that directly and indirectly correlates with depression. It predicts and interprets the binding affinity and interacting amino acids, further the study was continued with the prediction of Lipinski rule of five, bioactive scores, drug likeness score and toxicity profile of biochanin-A.

Materials and Methods

Docking studies:

Selection of molecular targets for antidepressant activity:

(a) *Monoamine Oxidase-A* (*MAO-A*):

MAO-A relates to the flavin adenine dinucleotide (FAD) – dependent amine- oxidoreductase enzyme class and catalyzes the oxygen- driven conversion of amines to their corresponding imines (Kalgutkar *et al*., 2001). In the brain, MAO-A is expressed in neurons and glial cells but with varying abundance (Saura *et al*., 1996). In the CNS, MAO'S are involved in the metabolism of classical monoaminergic neurotransmitters and play a key role in regulating their physiological functions (Cesura and Pletscher, 1992; Haefely *et al*., 1992; Kalgutkar *et al*., 2001). Thus MAO's are area of interest in neuropsychopharmacology, specifically in depression and parkinsonism.

(b) *Leucine Transporter* (*Leu T*):

In human Leu T_{Aa} is Na^+ and Cl^- concentration dependent and belong to Na^+/Cl^- dependent transporter from the solute carrier 6 (SLC6) family also known as neurotransmitter sodium symporters, or NSS. The main role is to transport many biologically important monoamines, for example dopamine (DA), noradrenaline and serotonin and a key target for the treatment of central nervous disorders (Wang and Lewis, 2010).

Inflammation initiates the tryptophan-kynurenine metabolism pathway. Circulating kynurenine is transported into the brain at the vascular blood–brain barrier (BBB) by the large amino-acid transporter LAT-1, where it is further metabolized into neurotoxic metabolites responsible for depressive symptoms. LAT-1 transports tryptophan, kynurenine and the amino acid leucine (Walker *et al*., 2015).

(c) *Serotonin* (*SERT*), *Norepinephrine* (*NET*), *Dopamine* (*DAT*) *Transporters*:

Monoamine transporters like the serotonin transporter (SERT), norepinephrine transporter (NET) and dopamine transporter (DAT) play a promising role in regulating the concentration of biogenic amines (serotonin, norepinephrine, dopamine) in the central nervous system (CNS) by transporting monoamines across neuronal membranes into presynaptic nerve cells. Thus they play a major role in depression (Susanna Nencetti *et al*., 2011).

(d) *Cycloxygenase -2* (*COX-2*):

Cyclo-oxygenase-2 (COX-2) is the indispensable enzyme for the production of a string of inflammatory cytokines. It balances the generation of cyclic adenosine monophosphate (cAMP), elevate the intracellular calcium ion concentration, activate phosphatidyl inositol 3-kinase, it regulates release of nerve growth factors in the brain. It promotes the production of inflammatory cytokines and induces the activity of nitric oxide synthase, which mediates the brain's nerve toxicity or protection. There is a close association between the cAMP transduction pathway and depression. The cAMP pathway is complex in neuron survival and support synaptic plasticity, and it is a common target for certain antidepressants. The increase in COX-2 expression affects not only the inflammatory response in the central nervous system by regulating activities of the cAMP system by activating prostaglandin E_2 receptor (PGE2-EP1, EP2, EP3, and EP4) but also the neural plasticity (Qi Chen *et al*., 2016).

(e) Interluekin-2 (*IL-2*):

Interleukin-2 produced by activated T cells is a T-cell growth factor that plays a central role in the generation of an immune response and in maintenance of T-cell proliferation. *In vivo* immune response of major depression is due to *in vivo* over production of IL-2 and of monocytic interleukins (i.e., IL-Iβ, IL-6). These findings are compatible with the existence of an immune or inflammatory response in depression disorder with the hypotheses that depression related to inflammation, infection, or autoimmunity (Maes *et al*., 1995).

(f) *Interleukin 1- β* (*IL-1β*) *and NOD – Like Receptor Protein* 3 (*NLRP3*):

The inflammasome is the intracellular multiprotein complex that controls the conversion of inactive pro-IL -1β into IL-1β, and considered as initial triggering complex of the inflammatory cascade, both centrally and peripherally, in animal models of MDD-I. Several types of inflammasomes are identified, the most investigated being the NOD-like receptor 3 (NLRP3) inflammasome, which is an important regulator of immune and central inflammatory processes. Various factors act to regulate the NLRP3 inflammasome, including reactive oxygen species (ROS), leading to caspase-1 induction, which cleaves pro-IL-1β and pro-IL-18 to their active proinflammatory forms (Ellul *et al*., 2016).

(g) *TNF-Receptor Associated Factor-2* (*TRAF-2*):

TRAF-2 regulates cellular response to cytokines/ stress signaling pathways and drives cell growth and death through the regulation of key stress-signaling cascades. It activates NF-KB through TNF-α-dependent induction of JNK activity. TRAF-2 play a critical role in the response of downstream signaling events to cytokine and stress conditions (Hasem *et al*., 2002).

(h) *Matrix Mettalopeptidase* (*MMP9*):

In depression and somatic diseases inflammatory process is considered as a frequent comorbidity. Study reported the participation of matrix metallopeptidases (MMPs) in the development and modulation of the inflammatory process. Inflammatory diseases affect neurodegenerative processes of the central nervous system (CNS) by influencing damage to the blood-brain barrier (BBB). MMPs play an important role in pathological processes in the CNS . Depression is a systemic inflammatory disease and MMPs are involved in the modulation of inflammatory processes and contribute for developmental and regenerative processes such as neurogenesis, axonal growth and regeneration, and myelin formation. In addition, MMP-9 in plasma is indicated to be one of the strongest markers of major depression (Kinga *et al*., 2016).

(i) *Phospholipase A2* (*PLA2*):

Phospholipase A2 (PLA2) and cyclooxygenase 2 (COX2) are the important enzymes and play an crucial role in cytokine-induced depression and sickness behavior (Kuan-Pin *et al*., 2010). Genetic variations in the COX2 and PLA2 genes gives rise to IFN-α induced depression, by affecting the levels of EPA and DHA. In addition, PLA2 genotype

is related with somatic symptoms in depression. Studies reported that cytokine treatment is associated with severe psychiatric symptoms, including depression, fatigue, anxiety and irritability. IFN-α induced psychiatric symptoms are similar to the symptoms of major depression, mainly the somatic symptoms studies are focused on the role of inflammation in depression and particularly in the mechanisms leading to somatic symptoms (Kuan-Pin *et al*., 2010).

(j) *Interleukin-1 Receptor- Associated Kinase 4* (*IRAK4*):

Interleukin 1 receptor (IL-1R)-associated kinase-4 (IRAK-4) is essential for various responses induced by IL-1R and Toll-like receptor signals. Study shows that the kinase activity of IRAK-4 is essential for the optimal transduction of IL-1-induced signals, including the activation of IRAK-1, NF-kappa B, and JNK, and the maximal induction of inflammatory cytokines, indicating that IRAK-4 is an integral part of the IL-1R signaling cascade and involves in immune mediated inflammatory responses thus play a crucial role in inflammation mediated depression (Elizabeth *et al*., 2004).

(k) *N-Lysine Methyl Transferase SETD6* (*NF-KB*):

In memory-related nuclear factor-kB RELA methylation at lysine 310 SETD6 plays a crucial role and also mediates increases in H3K9me2 (histone H3 lysine 9 dimethylation) in the dorsal hippocampus and SETD6 knockdown mediates memory consolidation, alters gene expression patterns, and disrupts spine morphology. SETD6, upstream initiator of H3K9me2 changes in the hippocampus during memory consolidation. In the hippocampus, NF-kB is involved in the epigenetic control of long-term memory and fearmemory retrieval involves activation of the NF-kB pathway to mediate histone acetylation at gene promoters in the hippocampus. NF-kB plays a critical role in the epigenetic control of memory consolidation for mitigating the cognitive symptoms of psychiatric conditions related to traumatic memories such as post traumatic stress disorder (William *et al*., 2019).

(l) *Mitogen Activated Protein Kinase-14* (*MAPK-14*):

Serine/threonine kinase acts as an essential component of the MAP kinase signal transduction pathway. In this MAPK14 is mainly involved in the cascades of cellular responses evoked by extracellular stimuli such as proinflammatory cytokines or physical stress leading to direct activation of transcription factors, thus show pathophysiological role in depression (Gayle *et al*., 2020).

(m) *Nitric Oxide Synthase* (*NOS*):

Nitric oxide (NO) is a free radical with signaling functions in the central and peripheral nervous system. It is produced by nitric oxide synthase (NOS) during the conversion of L-arginine to citrulline. Neuronal NOS is located at the sites of neuronal proliferation and migration in the hippocampal dentate gyrus (DG), forebrain subventricular zone (SVZ), rostral migratory stream and olfactory bulb . nNOS-derived NO exerts a negative control on the neurogenesis in the adult SVZ and DG. NOS inhibitors have antidepressant-like properties under physiological conditions (Qi-Gang *et al*., 2007).

(n) *Glutathione S- Transferase* (*GSTP1*):

Glutathione S- Transferase is an important antioxidant that reduces the 4-Hydroxy-2- trans-nonenal (4HNE) a major end product of lipid peroxidation. Oxidative stress-induced neuroinflammatory response, mitochondrial dysfunction, neuroplastic deficits and intracellular signaling pathways define the interrelationship between oxidative stress and depression and anxiety disorders, providing a novel path for the treatment (Ying *et al*., 2014). Oxidative damage to macromolecules such as lipids, proteins and nucleic acids as a result of excessive ROS lead to neuronal dysfunction that is connected with the development of depression disorder, where antioxidant like GSTP1 have a pivotal role (Ying *et al*., 2014).

(o) *Superoxide Dismutase* (*SOD*), *Catalase* (*CAT*), *Glutathione Peroxidase* (*GPx*):

Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GSR) prevent oxidative damage to brain cells. Moreover, lower gene expressions of SOD1, SOD2, CAT and GPX1 were observed in patients with MDD. SOD, GPX and CAT are major antioxidant enzymes that metabolize ROS into less toxic molecules and chip oxidative stress to avert oxidative damage. SOD catalyzes the conversion of superoxide anions into hydrogen peroxide (H_2O_2) and oxygen (O_2). CAT catalyzes H_2O_2 into water and oxygen, which is reported to be increased in depressed patients. Oxidative stress induced DNA damage and lipid peroxidation are noticed in patients with MDD (Meng-Chang and Tiao-Lai, 2016).

(p) *Vascular Endothelial Growth Factor Receptor-2* (*VEGFR-2*):

Vascular endothelial growth factor receptor-2 a cellular mitogen a vascular growth factor and permeability regulator has a neurotrophic and neuroprotective potential both in the peripheral and central nervous system. It is involved in the neurogenesis and regulate the neuronal development, differentiation and formation of vessels in the brain by directly influencing Schwann cells, neuronal progenitor cells, astrocytes and microglia contribute to the development process of nerve tissue. It acts as a trophic factor by influencing both vascular endothelial cells and brain cells. Antidepressant drugs are shown to induce hippocampal expression of VEGF and VEGFR2 signaling which is essential for cellular and behavioral response to antidepressant drugs (, and Ewa 2012).

(q) *Heat Shock Protein* (*HSP70*):

Heat shock proteins (HSPs) are regulatory molecular chaperones which play an important role for the maintenance of cellular homeostasisand neuronal protection. These proteins are important part of the cell's machinery for protein folding and protect cells from stress. It regulates the activity of glucocorticoid receptors, which play an important role in depression, and are new therapeutic targets for behavioral and psychological disorder including depression (Naoya *et al*., 2017).

(r) *Peroxisome Poliferator Activated Receptor -γ* (*PPAR- γ*):

The activation of microglia, macrophage and dendritic cells by toll-like receptors, CD40 or cytokine associated NF-κB pathway cause secretion of inflammatory cytokines resulting in pathogenesis of CNS diseases. PPAR agonists ligand activated transcription factor is a nuclear family receptor inhibit NF-κB pathway, further inhibits the release of proinflammatory cytokines and tends to inhibit CNS diseases (Bright *et al*., 2008).

(s) *Brain Derived Neurotrophic Factor* (*BDNF*):

Neurogenesis and neuroplasticity are promised in depression, with ensuring neurodegradation (Leonard and Maes, 2012). Stress alters the number and shape of neurons and glial cells in the brain regions of depressed patients and decreases proliferation of neural stem cells**.** Brain – Derived Neurotrophic factor (BDNF) is the most ample and broadly distributed neurotrophin in the central nervous system, involved in neuronal survival, growth and proliferation. BDNF is crucial for hippocampal plasticity and patients with depression has declined hippocampal volumes, due to glucocorticoid induced impairment of BDNF expression (Kaymak *et al*., 2010; Malykhin *et al*., 2010; Nifosi *et al*., 2010).

Preparation of target proteins:

All the ligand compounds were docked to the selected target proteins from *Homo sapiens*. The structures of SERT (SC- PDB ID:5I6X), NET (SC-PDB ID:4XP4), DAT (SC- PDB ID: 4M48), MAO-A (SC-PDB ID: 4COX), LeuT (SC-PDB ID: 3GWU), HSP70 (SC- PDB ID: 3JXU), NF-KB (SC- PDB ID: 3QXY), MAPK-14 (SC- PDB ID: 1DI9), VEGFR2 (SC-PDB ID: 1Y6B), TRAF2 (SC- PDB ID:1CA4), IL-2 (SC- PDB ID: 1M49),IL-1B (SC- PDB ID:9ILB), NLRP3 (6NPY),COX2 (SC- PDB ID: 4COX), PLA2 (SC- PDB ID: 1BD4), IRAK4 (SC- PDB ID: 2NRU), NOS2 (SC- PDB ID:3HR4), GSTP1(SC- PDB ID: 18GS), PPAR Gamma (SC- PDB ID: 1I7I), MMP9

(SC- PDB ID: 1GKC), SOD (SC- PDB ID:2ADQ), CAT (SC- PDB ID:1DGF),GPx (SC- PDB ID:2I3Y), BDNF (SC-PDB ID : 1BND) were obtained from database available in 1- click docking of MCULE software.

Preparation of ligands:

The structures of native ligands from each target macromolecules were prepared to separate from the proteins, water and miscellaneous substances. The structures of the ligands (Biochanin-A, imipramine, fluoxetine, diclofenac, ibuprofen) were sketched in MCULE software.

Molecular docking:

Molecular docking of compounds with the 3D X-ray crystal structure of human SERT (SC- PDB ID:), NET (SC- PDB ID:), DAT (SC- PDB ID:), MAO-A (SC-PDB ID: 4COX), LeuT (SC-PDB ID: 3GWU), HSP70 (SC- PDB ID: 3JXU),NF-KB (SC- PDB ID: 3QXY), MAPK-14 (SC- PDB ID: 1DI9), VEGFR2 (SC- PDB ID: 1Y6B), TRAF2 (SC- PDB ID:1CA4), IL-2 (SC- PDB ID: 1M49),IL-1B (SC- PDB ID:), COX2 (SC- PDB ID: 4COX), PLA2 (SC- PDB ID: 1BD4), IRAK4 (SC- PDB ID: 2NRU), NOS2 (SC- PDB ID:3HR4), GSTP1(SC- PDB ID: 18GS), PPAR Gamma (SC- PDB ID: 1I7I), MMP9 (SC- PDB ID: 1GKC), SOD (SC- PDB ID), CAT (SC- PDB ID), GPx (SC- PDB ID) were imported into MCULE, the online drug discovery platform. The structure of title compounds were drawn using Chemsketch 12.0 software available in MCULE and run the docking for a selected target. Consequently it generates the different pose of ligands with the target and among those we selected the best pose of ligand, which gained the good score.

Docking method validation:

To make sure that the docking studies were well performed and represented the reasonable potential binding model, the docking methods and parameters used were validated by redocking experiment. Each copy of native ligand was docked into the native protein to set on the ability of the program to reproduce the orientation and position of the ligand observed in the crystal structure.

Lipinski's analysis:

Lipinski's rule evaluates drug likeness and determines the pharmacological activity. The Lipinski's analyses properties like molecular weight, log P, number of hydrogen bond donors and acceptors. Lipinski's parameters were retrieved for ligand using PubChem tool (Lipinski, 2001).

Molecular property prediction:

Molecular properties like log P (lipophilicity), total polar surface area (TPSA), number of hydrogen bond donors (HBD) and acceptors (HBA), number of rotatable bonds and as well as prediction of bioactivity score for the important drug targets like GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors were assessed by the online tool kit Molinspiraton (www.molinspiraton.com). The Osiris Property Explorer is a vital Actelion's in-house substance registration system. It calculates the various drug relevant properties such as log S (solubility), drug likeness score and toxicity such as mutagenicity, tumorogenicity, irritant and reproductive effects (Ertl *et al*., 2000).

Results and Discussion

Biochanin-A and standard antidepressant drugs imipramine and fluoxetine, phenelzine, bupropion were subjected to *in silico* docking analysis by using the commercial docking software MCULE, 1-click docking to predict the anti-depressant potential. All the compounds were docked against their respective antidepressant targets Monoamine Oxidase-A (MAO-A), Serotonin Transporter (SERT), Norepinephrine Transporter (NET), Dopamine Transporter (DAT), Leucine Transporter (Leu T), Cycloxygenase -2 (COX-2), Interluekin 1 Beta (IL-1β), Nod-Like Receptor-3 (NLRP3), Interluekin-2 (IL-2), TNF -Receptor Associated Factor-2 (TRAF2), Matrix Metallopeptidase (MMP9), Phospholipase A2 (PLA2), Interleukin-1 Receptor- Associated Kinase 4 (IRAK4), Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells (NF-KB), Mitogen Activated Protein Kinase- 14 (MAPK-14), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2),

Heat Shock Protein (HSP70), Peroxisome Proliferator Activated Receptor – Γ (PPAR- γ), Brain-Derived Neurotrophic Factor (BDNF), Nitric Oxide Synthase (NOS), Glutathione S-Transferase (GSTP1), Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase (GPx).

The goal of molecular (ligand and receptor) docking is to predict the principal binding model of a ligand with a protein of known three dimensional structures (Mittal *et al*., 2009). Binding modes of bioactive compounds in the binding site of target proteins, intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the receptor ligand complexes (Srivastava *et al*., 2010). Lipinski's rule five is salient for drug development where a pharmacologically active phytocompounds should have not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular weight under 500 dalton, Partition coefficient A log P should be less than 5. Biochanin-A satisfied the Lipinski's properties. The Biochanin-A had the potential to dock with the target proteins and their interaction details are listed in (Tables 1-4).

SERT, NET, DAT, MAO-A, Leu-T are major antidepressant targets in treating depression. In the CNS, MAO'S are involved in the metabolism of classical monoaminergic neurotransmitters and play a key role in regulating their physiological functions (Cesura and Pletscher, 1992; Haefely *et al*., 1992; Kalgutkar *et al*., 2001). The target proteins SERT, NET, DAT, MAO-A, Leu-T when counteracted with the standard antidepressant drugs like fluoxetine, desipramine, buproprion, phenelzine and imipramine exhibited a least score of -6.5, -6.7, -5.1,-6.1, -6.5 Kcal/mol, respectively. whereas the biochanin-A exhibited least docking score with energy values of -7.3, -6.6, -6.2, -9.0,-7.2 Kcal/mol, respectively. The results indicate that biochanin-A is having highest binding affinity than that of respective standard marketed drugs. It is interesting to note that biochanin-A showed potent binding affinity towards the MAO-A (-9.0) based on their docking scores than the standard phenelzine (MAO inhibitor), which is in accordance with the previous study reports stating that biochanin –A may be a novel MAO inhibitor (Guoxin *et al*., 2005).

Inflammation and depression are interconnected and previous study evidenced the role of inflammatory markers in the depression. (Qi Chen *et al*., 2016).Cyclooxygenase-2 (COX2) is an indispensable enzyme for the production of a string of inflammatory cytokines. it plays an indirect key role in depression via cAMP pathway. The cAMP pathway is complexed in neuron survival and support synaptic plasticity, and it is a common target for certain antidepressants. (Qi Chen *et al*., 2016). *In vivo* overproduction of IL-2 and of monocytic interleukins (i.e., IL-Iβ, IL-6) were reported in major depression.These findings are in concurrence with the hypotheses that depression related to inflammation, infection, or autoimmunity (Maes *et al*., 1995).

Studies also evidenced the role of IL-1β and NOD- like receptor 3 protein (NLRP3) as a novel targets to depression as these inflammasomes are important regulators of immune and central inflammatory processes (Ellul *et al*., 2016). Phospholipase A2 (PLA2) is another major enzyme as cyclo-oxygenase 2 (COX-2) in the metabolism of polyunsaturated fatty acids, and play a crucial role in cytokine-induced depression and sickness behavior (Kuan-Pin *et al*., 2010). IRAK-4 is an integral part of the IL-1R signaling cascade and involves in immune mediated inflammatory responses thus play a crucial role in inflammation mediated depression (Elizabeth *et al*., 2004).

TRAF2 serves as a central regulator of the cellular response to cytokines and stress signaling pathways and control cell growth and death through the regulation of key stress-signaling cascades. It activates NF-KB through TNF-a-dependent induction of JNK activity. Availability of TRAF2 is therefore expected to play a critical role in the response to downstream signaling events to cytokine and stress conditions (Hasem *et al*.,

Table 1: Molecular docking scores and interacting amino acids of biochanin-A and docking scores of standards with Monoamine Markers

S. No.	TARGET PROTEINS	PDBID	BIOCHANIN-A		STANDARDS	DOCK SCORE (Kcal/Mol)
			Dock score	Interacting Amino acids		
1	Monoamine oxidase A (MAO-A)	2BXR	**-9.0**	ILE 316 TYR 188	Phenelzine	-6.1
2	Serotonin Transporter (SERT)	5I6X	-7.3	GLU 65 GLY61	Imipramine Fluoxetine	-6.8 -6.5
3	Norepinephrine transporter (NET)	4XP4	-6.6	SER 44 PRO 45	Imipramine Fluoxetine desipramine	-6.6 -6.6 -6.7
4	Dopamine transporter (DAT)	4M48	-6.2	PRO 615 GLY 516	Bupropion	-5.1
5	Leucine transporter (Leu T)	3GWU	-7.2	PHE 390 VAL29	Imipramine fluoxetine	-6.5 -7.2

Table 2: Molecular docking scores and Interacting Amino acids of biochanin-A and docking scores of standards with Inflammatory Markers

S. No.	TARGET PROTEINS	PDBID	BIOCHANIN-A		STANDARDS	DOCK SCORE (Kcal/Mol)
			Dock score	Interacting Amino acids		
6	Cyclooxygenase (COX-2)	4COX	**-9.2**	ILE 314 GLU 315	Imipramine Fluoxetine Celecoxib	-7.9 -9.1 -8.2
7	Interleukin 2 (IL-2)	1M49	-6.9	LUE 39 ASR 68	Imipramine Fluoxetine Diclofenac	-6.1 -5.9 -5.5
8	Interleukin 1β (IL-1β)	91LB	-5.5	LEU 134 LEU 22	Imipramine Fluoxetine Diclofenac	-5.1 -5.3 -5.4
9	Nod like receptor 3 protein (NLRP3)	6NPY	-7.4	CYS 298 THR 299	Imipramine Fluoxetine Diclofenac	-6.6 -7.0 -6.9
10	TNF -Receptor Associated Factor-2(TRAF2)	1CA4	-6.2	GLY 135 LEU 76	Imipramine Fluoxetine Diclofenac	-5.3 -6.0 -6.2
11	Matrix Mettalopeptidase (MMP9)	1GKC	**-9.1**	TYR 106 MET 35	Imipramine Fluoxetine Diclofenac	-6.7 -8.5 -7.3
12	Phospholipase A2 (PLA2)	1DB4	-7.8	VAL 50 GLY 29	Imipramine Fluoxetine Diclofenac	-7.3 -7.2 -7.9
13	Interleukin-1 Receptor- Associated Kinase 4 (IRAK4)	2NRU	**-8.4**	LYS 28 GLT 229	Imipramine Fluoxetine Diclofenac	-7.5 -7.7 -7.7
14	Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells (NFKB)	3QXY	**-8.9**	GLU 33 ARG 253	Imipramine Fluoxetine Diclofenac	-8.3 -8.6 -8.3
15	Mitogen Activated Protein Kinase- 14 (Mapk-14)	3KF7	-7.6	ALA 47 GLY 106	Imipramine Fluoxetine diclofenac	-6.5 -7.6 -7.1

Table 3: Molecular docking scores and Interacting Amino acids of biochanin-A and dock scores of standard with Oxidative stress Markers

S. No.	TARGET PROTEINS	PDBID	BIOCHANIN-A		STANDARDS	DOCK SCORE (Kcal/Mol)
			Dock score	Interacting Amino acids		
16	Nitric Oxide Synthase (NOS2)	3HR4	-9.2	ASP87 THR32	Imipramine Fluoxetine Ascorbic acid	-8.1 -8.5 -5.5
17	Glutathione S-Transferase (GSTP1)	18GS	-7.8	ASP97 CYS100	Imipramine Fluoxetine Ascorbic acid	-4.2 -5.1 -4.7
18	Superoxide Dismutase (SOD)	2ADQ	-6.0	LEU111 THE110	Imipramine Fluoxetine Ascorbic acid	-5.5 -5.0 -4.3
19	Catalase CAT	1DGF	-7.2	PRO70 ARG365	Imipramine Fluoxetine Ascorbic acid	-6.4 -7.1 -5.1
20	Glutathione Peroxidase GPx	213Y	-5.7	LUE97 VAL96	Imipramine Fluoxetine Ascorbic acid	-5.6 -5.7 -4.2

Table 4: Molecular docking scores and Interacting Amino acids of biochanin-A and dock scores of standards with Selective Markers

S. No.	TARGET PROTEINS	PDBID	BIOCHANIN-A		STANDARDS	DOCK SCORE (Kcal/Mol)
			Dock score	Interacting Amino acids		
21	Vascular Endothelial Growth Factor Receptor-2 (VGFR2)	1Y6B	**-8.3**	ARG150 ARN101	Imipramine Fluoxetine	-7.4 -7.5
22	Heat Shock Protein(HSP 70)	3JXU	**-9.2**	GLY98 LUE197	Imipramine Fluoxetine	-7.5 -8.1
23	Peroxisome Poliferator Activated Receptor – γ(PPARγ)	1I7I	**-8.2**	VAL126 GLY56	Imipramine Fluoxetine	-7.2 -7.1
24	Brain-derived Neurotrophic Factor (BDNF)	1BND	-6.3	CYS109 VAL110	Imipramine Fluoxetine	-4.4 -5.9

2002). MMPs play a crucial role in pathological processes in the CNS . Since depression is a systemic inflammatory disease and MMPs are involved in the modulation of inflammatory processes and its role is interesting in course of depression (Kinga *et al*., 2016). MAPK14 is mainly involved in the cascades of cellular responses evoked by extracellular stimuli such as proinflammatory cytokines or physical stress leading to direct activation of transcription factors, thus show pathophysiological role in depression (Gayle *et al*., 2020).

Onset of depression is related to a variety of pro-inflammatory cytokines and inflammatory mediums (Qi Chen *et al*., 2016). Depression and inflammation are interconnected, we selected these inflammatory target proteins to investigate the potency of biochanin-A as an invention of preventive drug for depression.

The target proteins COX-2, IL-2, IL-1β, NLRP3, TRAF2, MMP9, PLA2, IRAK4, NFKB, MAPK14 when counteracted with the standard anti-inflammatory drugs like celecoxib for COX-2 (-10.3), Diclofenac exhibited a least score of -8.2, -5.5, -5.4, -6.9, -6.2, -7.3, -7.9, -7.7, -8.3 , -7.6Kcal/mol and for and ibuprofen -7.9, -5.6, -5.2, -6.2, -4.9, -8.1, -7.0, -7.0, -7.6, -7.0 Kcal/mol, respectively. whereas the biochanin-A exhibited least docking score with energy values of -9.2, -6.9, -5.5, -7.4, -6.2, -9.1, -7.8, -8.4, -8.9, -7.6 Kcal/mol, respectively. The results indicate that biochanin-A is having closer binding affinity as the standard celecoxib (-10.3) and showed highest binding affinity than that of respective standard marketed drugs diclofenac and ibuprofen. It is interesting to note that biochanin-A showed potent binding affinity towards the COX-2(-9.2), MMP9 (-9.1) NFKB (-8.9) and IRAK4 (IL-1) (-8.4) based on the docking scores diclofenac and ibuprofen and may be a better alternative to treat inflammation mediated depression.

SOD, GPX and CAT are major antioxidant enzymes that metabolize ROS into less toxic molecules and chip oxidative stress to avert oxidative damage. Oxidative stress is connected with DNA damage and lipid peroxidation, and are also noticed in patients with MDD (Meng-Chang and Tiao-Lai, 2016). Glutathione S- Transferase reduces the 4-Hydroxy-2- trans- nonenal (4HNE) a major end product of lipid peroxidation. Brain oxidative stress disturbances might be a plausible pathogenesis and risk factor for several specific diseases of the nervous system including behavioral disturbances and disorders (Ying Xu *et al*., 2014). nNOS-derived NO exerts a negative control on the neurogenesis in the adult forebrain subventricular zone (SVZ) and hippocampal dentate gyrus (DG). NOS inhibitors have antidepressant-like properties under physiological conditions (Qi-Gang *et al*., 2007).

In this study we docked biochanin- A with selected oxidative stress markers. The target proteins NOS2, GSTP1, SOD, CAT, GPx, when counteracted with the standard antioxidant like ascorbic acid exhibited a least score of -5.5, -4.7, -4.3, -5.1, -4.2 Kcal/mol, respectively. whereas the biochanin-A exhibited least docking score with energy values of -9.2, -7.8, -6.0, -7.2, -5.7Kcal/mol, respectively. The results indicate that biochanin-A is having higher binding affinity than that of respective standard ascorbic acid. Results of docking revealed that biochanin-A exhibited potent binding affinity towards the NOS2(-9.2) indicating biochann-A is a potent NOS inhibitor and may exert potent antidepressant properties.

The activation of microglia, macrophage and dendritic cells by toll-like receptor, CD40 or cytokine associated NF-κB pathway cause secretion of inflammatory cytokines resulting in the pathogenesis of CNS diseases. PPAR agonists inhibit NF-κB pathway, thereby inhibits CNS diseases (Bright *et al*., 2008). Neurogenesis and neuroplasticity are promised in depression, with ensuring neurodegradation (Leonard and Maes, 2012). Vascular endothelial growth factor receptor-2 a cellular mitogen a vascular growth factor and permeability regulator has a neurotrophic and neuroprotective potential both in the peripheral and central nervous system. Antidepressant drugs are shown to induce hippocampal expression of VEGF and VEGFR2 signaling which is essential for cellular and behavioral responses to antidepressant drugs (Marta and Ewa, 2012).

BDNF located in the cerebral cortex and hippocampus is a key regulator for neuronal plasticity regulation, and very important factor as neuroprotectant against injury and promote neuronal regeneration.. The expression of BDNF is closely related to the cAMP signaling pathway and has a key role in depression (Qi Chen *et al*., 2016). Heat shock proteins (HSPs), are regulatory molecular chaperones, play an important role for the maintenance of cellular homeostasisand neuronal protection. It regulates the activity of glucocorticoid receptors, which play an important role in depression and are new therapeutic targets for behavioural and psychological disorder including depression (Naoya *et al*., 2017).

Table 5: Bioactive scores of biochanin-A

Compound	GPCRL	ICM	KI	NRL	PI	EI
Biochanin-A	-0.23	-0.59	-0.07	0.23	-0.66	0.07

GPCRL: G protein coupled receptor ligand, ICM: ion channel modulator, KI: Kinase inhibitor, NRL: Nuclear receptor ligand, PI: Protease inhibitor, EI Enzyme inhibitor.

Table 6: *In silico* pharmacokinetics of biochanin-A

S. No.	PHARMACOKINETICS	RESULT
1	GI absorption	High
2	BBB permeant	Yes
3	p-gp substrate	No
4	CYP 1A2 Inhibitor	Yes
5	CYP 2C19 Inhibitor	No
6	CYP 2C9 Inhibitor	No
7	CYP 2D6 Inhibitor	Yes
8	CYP 3A4 Inhibitor	Yes
9	Log K_P (Skin Permeation)	-5.91 cm/s

Based on the above studies, we selected VGFR2, HSP 70, PPARγ, BDNF targets and docked against biochanin-A and marketed standard antidepressants like imipramine and fluoxetine. Results showed docking score of standard antidepressants imipramine as -7.4, -7.5, -7.2, -4.4Kcal/mol, respectively and for fluoxetine -7.5, -8.1, -7.1, -5.9 Kcal/mol, respectively. Biochanin-A showed docking scores of -8.3, -9.2, -8.2, -6.3, respectively. Based on results it was found that biochanin-A showed greater binding affinity towards HSP70 (-9.2), and VGFR2(-8.3) and PPARγ (-8.2) revealing its neuroprotective potential and can be a novel and potent target for the behavioural disorders specifically depression.

Bioactivity score prediction:

The bioactivity scores of the biochanin-A towards GPCR, kinase inhibitor, protease inhibitor and enzyme inhibitor mechanisms were calculated using Molinspiration programme and depicted in (Table 5). For average organic molecules the probability is that, if the bioactivity score is more than 0 then it is active; between -0.5 to 0 then moderately active (Verma *et al*., 2012). Table 5 shows that biochanin-A displayed more selectivity towards nuclear receptor ligand (0.23) enzyme inhibition (0.07) which gave additional support to the *in silico* result of predicted antidepressant activity.

In silico pharmacokinetics of Biochanin-A:

The pharmacokinetic profile of the molecule was analyzed *In silico* by Swiss ADME and depicted in (Table 6). Biochanin –A was predicted to have a high passive human gastrointestinal absorption (GI) and BBB permeability. At the same time, we observed that the permeability glycoprotein (P-gp), considered for the efflux through biological membranes and the prediction documented that Biochanin-A was not a substrate for P-gp. Biochanin-A was also predicted to inhibit the major CYP isoforms (CYP1A2, CYP2D6, and CYP3A4), which are important in drug elimination through metabolic transformation.

Molecular properties prediction:

The efficacy of any drug depends on its high oral bioavailabilty in human beings. For any compound to become a successful drug candidate it should satisfy the Lipinski's rule of five. This rule predicts the molecular properties which are important for drug pharmacokinetics (ADME) within the human

Table 7: Molecular descriptors of Biochanin- A

S. No.	Molecular descriptors	Values
1	Log P	2.80
2	Log S	-4.3
3	TPSA	79.90
4	%ABS	84.3
5	n- HBA	5
6	n- HBD	2
7	n- ROTB	2
8	MW	284.26
9	MUT	G
10	TUM	G
11	IRR	G
12	REPE	G
13	DL	6.35

Log P : Lipophilicity; Log S : Solubility; TPSA: Total polar surface area; n-HBA: No of hydrogen bond acceptors; n-HBD: No of hydrogen bond donors; n- Violations; n-ROTB: No of rotatable bonds; MW: Molecular weight; MUT: Mutagenic; TUM: Tumerigenic; IRR: Irritant; REPE: Reproductive effect; DL: Drug- likeness score; G: No Risk

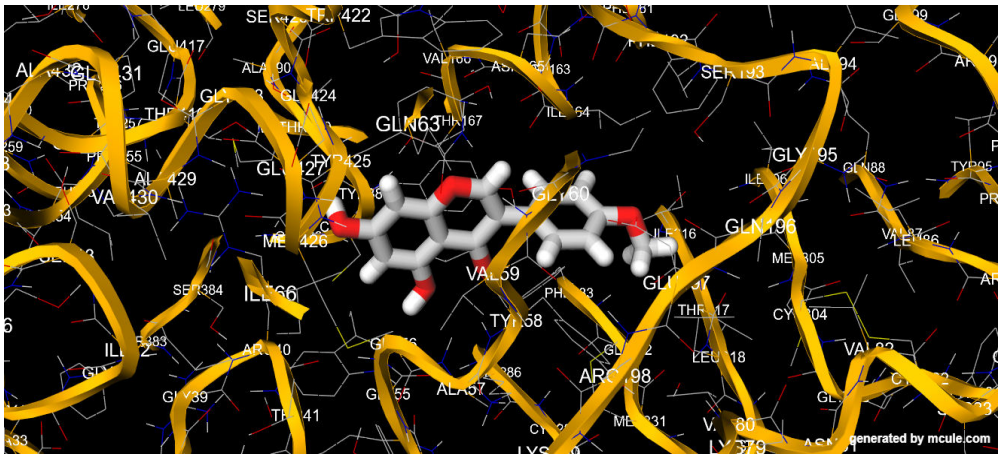

Fig. 1: Interaction of biochanin-A with MAO-A (-9.0).

body. According to Lipinski's rule of five any compound to become orally active drug, if it has a molecular weight not more than 500, partition coefficient (log P) below 5, number of hydrogen bond donors not more than 5 and number of hydrogen bond acceptors not more than 10. Topological polar surface area is another descriptor used to assess the drug transporter properties. TPSA is the sum of surfaces of polar atoms such as oxygen, nitrogen and attached hydrogen. The % of absorbance was calculated by the equation:

$$\% \text{ ABS} = (109 - 0.345) \times \text{TPSA}$$

Furthermore, the number of rotatable bonds is important for a conformational flexibility of the molecule in turn decides the binding of receptors. Along with Lipinski's rule of five, these two parameters i.e. the number of rotatable bonds (not more than 10) and polar surface area (not more than 140 A^0) is essential for a good oral bioavailabilty (Verber, 2002).

The study was further continued with the prediction of molecular properties and toxicity profile of the biochanin-A by using MOLINSPIRTION and OSIRIS tool kits (Table 7). Results shown biochanin-A obeys the Lipinski rule of five and free from major toxicities.

Biochanin-A exhibited good to moderate

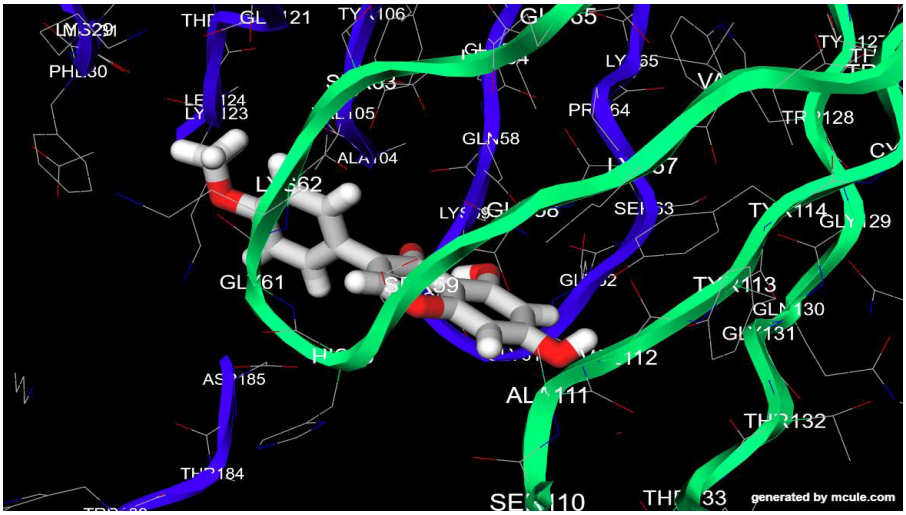

Fig. 2: Interaction of biochanin-A with SERT.

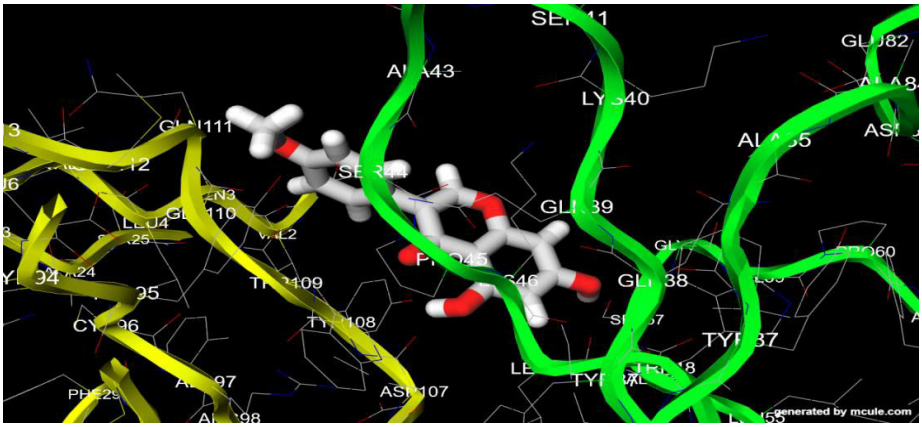

Fig. 3: Interaction of biochanin-A with NET.

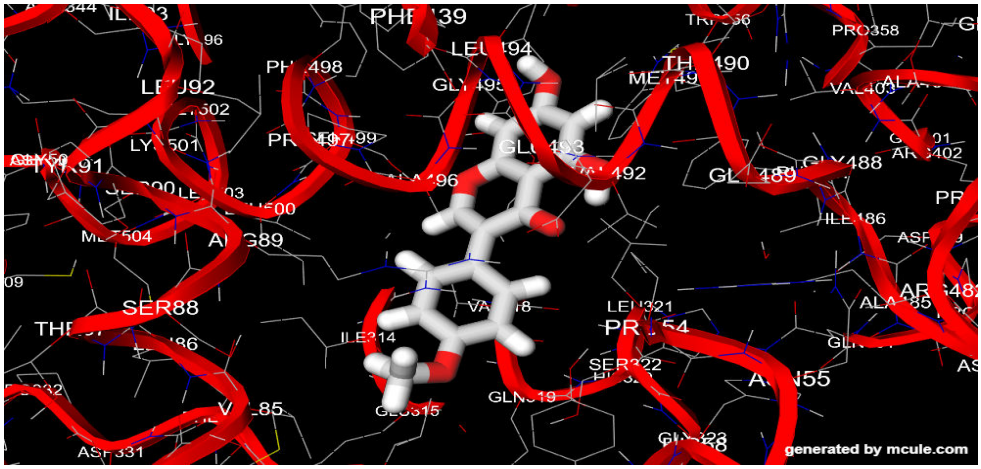

Fig. 4: Interaction of biochanin-A with COX-2(-9.2).

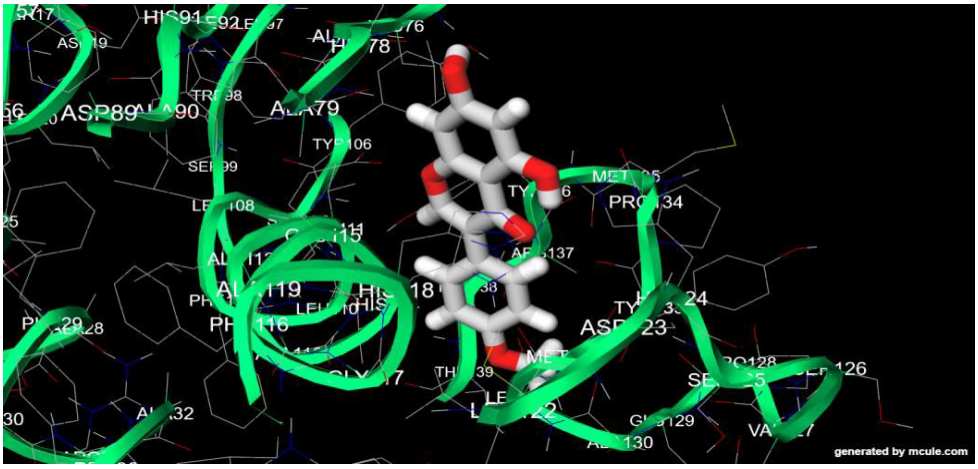

Fig. 5: Interaction of biochanin-A with MMP9 (-9.1).

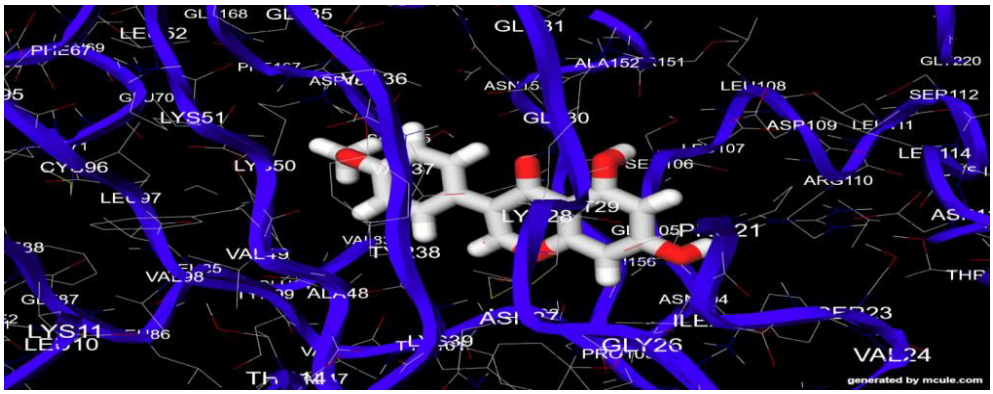

Fig. 6: Interaction of biochanin-A with IRAK4 (-8.4).

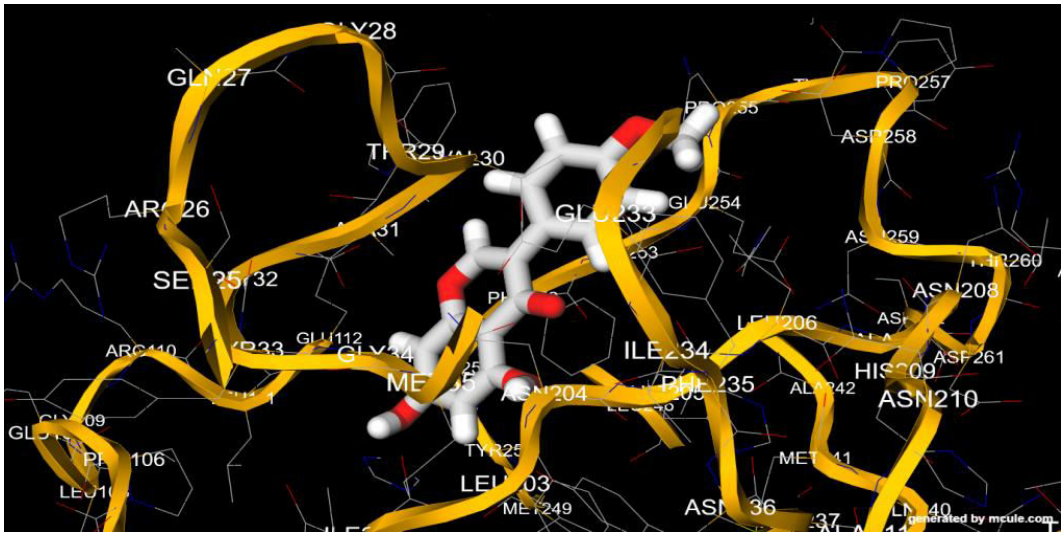

Fig. 7: Interaction of biochanin-A with NF-KB(-8.9).

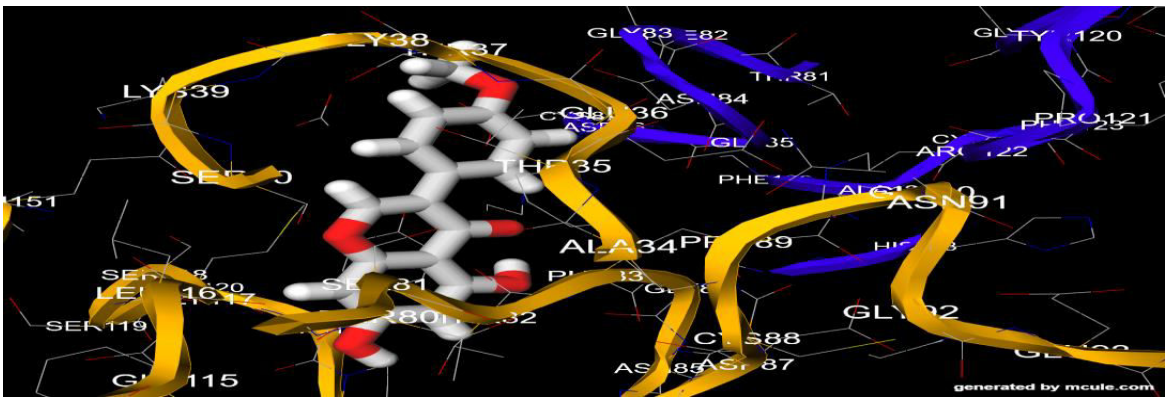

Fig. 8: Interaction of biochanin-A with NOS (-9.2).

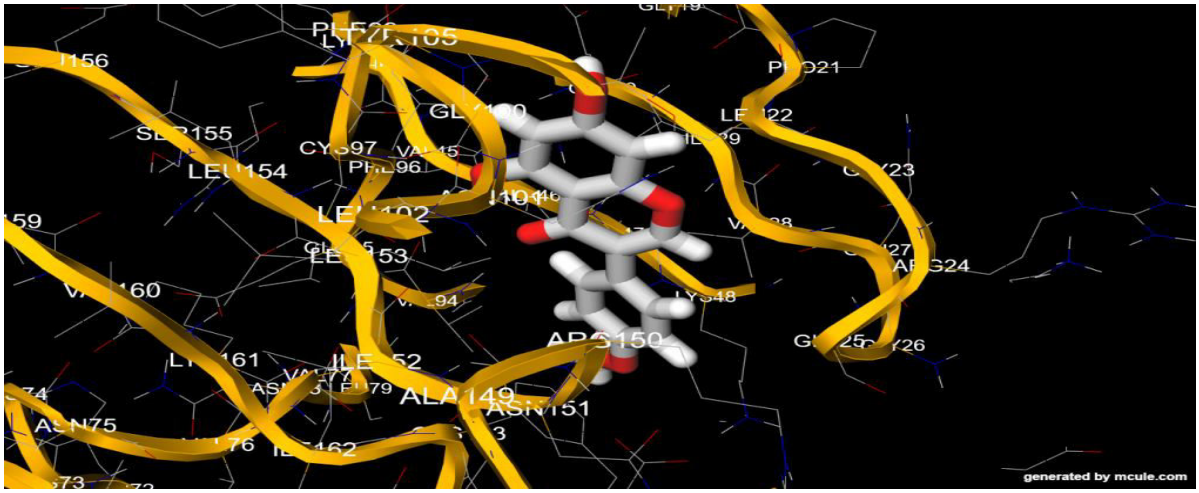

Fig. 9: Interaction of biochanin-A with VEGFR2 (-8.3).

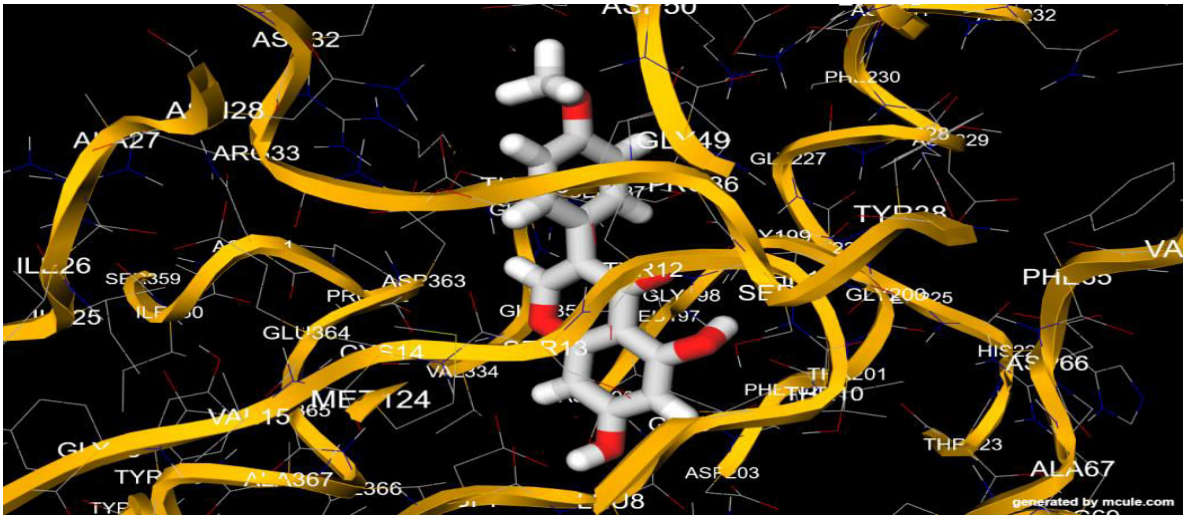

Fig.10: Interaction of biochanin-A with HSP70 (-9.2).

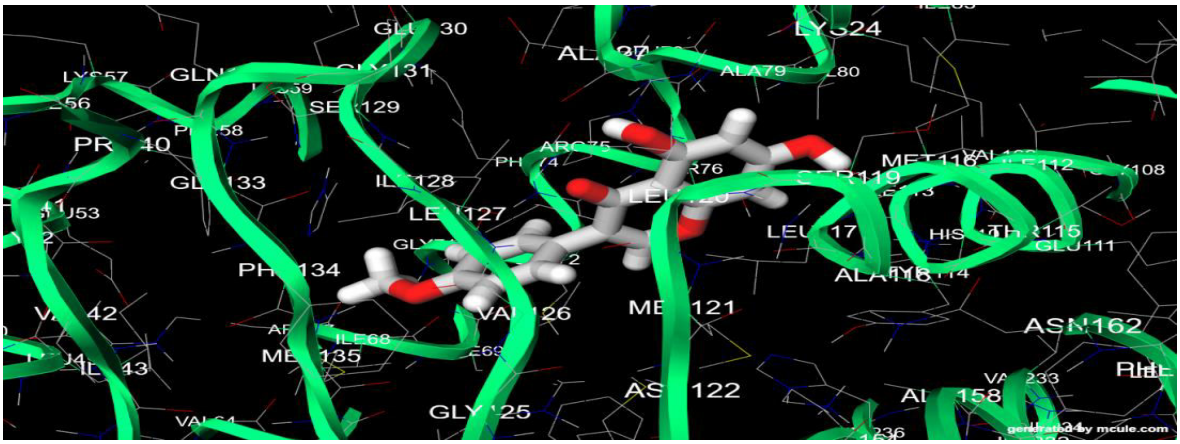

Fig. 11: Interaction of biochanin-A with PPAR- γ (-8.2).

affinity towards all the 24 selected targets against depression assessed by using one click molecular docking tool. Among all targets biochanin-A showed highest docking score towards NOS2 (-9.2) compared to imipramine (-8.1), fluoxetine (-8.5) and ascorbic acid (-5.5), HSP70(-9.2) whereas the standard imipramine (-7.5), fluoxetine (-8.1), COX-2 (-9.2) and standards imipramine (-7.9), fluoxetine (-9.1), MMP9 (-9.1) standard imipramine (-6.7) and fluoxetine (-8.5), MAO-A (-9.0) standard phenelzine (-6.1), NFKB (-8.9) standards imipramine (-8.3) and fluoxetine (-8.6), and diclofenac (-8.3) IRAK4 (-8.4) standards imipramine (-7.5) and fluoxetine (-7.7), PPAR-γ (-8.2) standards imipramine (-7.2) and fluoxetine (-7.1), SERT(-7.3) standards imipramine (-6.8) and fluoxetine (-6.5), NET (-6.6) standard desipramine (-6.7) DAT (-6.2) and standard bupropion (-5.1) given in (Figs. 1-11). The anti-depressant activity of biochanin-A was in correlation with molecular docking studies. Moreover biochanin-A obey the Lipinski rule of five, with good percentage of oral absorption and have good bioactive score.

Conclusion

The present study indicates that biochanin-A follows Lipinski's rule of five with good percentage of oral absorption and free from toxicity and expected to be an active component as a drug. The results obtained from the docking studies showed that biochanin-A has a good binding affinity with all antidepressant, proinflammatory and oxidative stress markers. ADMET showed the molecular properties of biochanin-A which support the fact that it becomes a lead drug. As major proteins selected for docking are proinflammatory and anti-inflammatory mediators involved in neuroinflammation. This *in silico* study is actually an additional advantage to the screening, the inflammatory mediator's inhibitors as antidepressants are closely linked with neuroinflammation. Further *in vivo* studies are essential to develop a lead molecule for the prevention and treatment of depression. Therefore, *in silico* study reveals that biochanin-A may act as a novel potent lead molecule against depression.

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