Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [5] April 2021 : 72-80

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

In Silico Screened Flavanoids of *Glycyrrhiza Glabra* Inhibit CPLA2 and SPLA2 in LPS Stimulated Macrophages

Avinash KO[1], Sushma Pradeep[2], Chandan Shivamallu[2], Gopenath TS[2], Kiran Kumar MN[2], Kanthesh BM[3], JR Kumar[1]*

[1]Division of Biochemistry, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru
[2]Department of Biotechnology and Bioinformatics, School of life sciences, JSS Academy of Higher Education and Research, Mysuru
[3]Division of Molecular Biology, School of Life sciences, JSS Academy of Higher Education and Research, Mysuru.

Corresponding Author's Email: kumarjr2013@gmail.com

ABSTRACT

*Phospholipases hydrolyze fatty acids from the **sn**-2 position of membrane phospholipids. In addition to their normal metabolic processes, phospholipases such as cytosolic phospholipase A2 (cPLA2) and secreted phospholipase A2 (sPLA2) are well known to generate active lipid metabolites that promote inflammatory metabolic diseases such as atherosclerosis, hyperlipidemia, obesity, etc. Inhibiting the activity of cPLA2 and sPLA2 might be very important to control inflammatory reactions. Several plant based molecules are being considered which possess inhibitory activity towards these phospholipases. Glycyrrhiza glabra, commonly called licorice is one of those medicinal herbs which are used to treat many diseases, such as respiratory disorders, epilepsy, hyperdipsia, fever and even jaundice. Mass spectrometric analysis of the plant revealed that it contains some important flavanoids such as Glycyrin, Isoliquiritin, Liquiritin, Formnonetien, Jaranol, Isoliquiritigenin and Glycyrrhizaisoflavone. In silico docking studies showed that these flavonoids interacted with both cytosolic PLA2 (cPLA2) and secretary PLA2 (sPLA2). Isoliquiritigenin was found to be more effective in binding to the active sites of cPLA2 and sPLA2 among all flavonoids tested. In vitro, Jaranol, Glycyrrhizaisoflavone and Isoliquiritigenin inhibited the function of cPLA2 and sPLA2 in macrophages induced by lipopolysaccharides in a concentration dependent manner, suggesting that these flavanoids possess anti-inflammatory and immunomodulatory functions. Further, they can be target drug molecules against cPLA2 and sPLA2.*

Key words: Phospholipases, flavanoids, *Glycyrrhiza glabra*, LPS, macrophages

Received 02.12.2020 Revised 11.03.2021 Accepted 22.03.2021

INTRODUCTION

Phospholipase A2 (PLA2) is a family of proteins consisting of five subtypes: cytosolic PLA2 (cPLA2), secreted PLA2, calcium-independent PLA2, acetylhydrolase platelet-activating factor (PAF), and lysosomal PLAs [1, 2]. Several research studies have focused on cPLA2 due to its preference for hydrolyzing membrane glycerophospholipids at position sn-2 to form arachidonic acid (AA) [3]. AA is transformed through the catalytic action of lipoxygenase or cyclooxygenase [4, 5] to active inflammatory lipid mediator eicosanoids, including leukotrienes (LTs), lipoxins, thromboxanes, and prostaglandins. The involvement of cPLA2 in the production of lipid mediators and their role in inflammation and cancer makes it a potentially significant pharmacological target. Despite its relevance, the cPLA2 down-regulation mechanisms have not yet been determined. A novel approach is required to understand the mechanisms of cPLA2 down-regulation to improve the treatment of inflammatory diseases.

Inflammation of the CNS is known to be mediated through the hydrolysis of fatty acids from the sn-2 position of membrane phospholipids by secretory phospholipase A2-IIA (sPLA2-IIA), leading to the generation of free arachidonic acid (AA), lysophospholipids, precursors of pro-inflammatory lipid mediators such as prostaglandins (PGs) and leukotrienes (LTs) [6, 7]. sPLA2 is also a most abundant isoform isolated from inflammatory fluids and cells, playing prominent roles in inflammatory and infectious diseases [8,9, 10,11]. The hippocampus and cerebral cortex of the ischemic and Alzheimer's Disease brains are also found to have significant amounts of sPLA2-IIA [12,13]. A crosstalk exists between the two phospholipases where sPLA2-IIA interacts with cytosolic phospholipase A2 (cPLA2) to supply AA because of its phospholipase enzymatic activity to downstream cyclooxygenase-2 (COX-2) for PGE2 biosynthesis [14]. However, the exact role of sPLA2-IIA in neuroinflammation is still unclear.

Pro-inflammatory enzymes and cytokines are more desired as therapeutic targets for a range of inflammatory diseases [6]. In this respect, the 14-18 kD secreted phospholipase A2s (sPLA2s) are of interest because of their availability in blood circulation and also because most types of inflammation are associated with local and systemic elevation of sPLA2s. This is evident from patients affected by chronic inflammatory disorders such as acute pancreatitis, septic shock, severe burns, and autoimmune diseases who have elevated levels of extracellular sPLA2s in plasma [7]. Phospholipases are also found to accumulate in inflammatory fluids such as the synovial fluid of rheumatoid arthritis patients, the bronchoalveolar lavage of bronchial asthma patients and the nasal secretions of allergic rhinitis patients. More recent experimental studies indicate that sPLA2s are involved in precipitated traumatic and autoimmune neurodegeneration and are thus also a possible target for the treatment of nervous system disorders due to excess release of neurotransmitters, possibly because of high phospholipid turnover, long-term potentiation and memory processes [15]. However, increased degradation of phospholipids due to activation of PLA2 isozymes contributes to changes in membrane permeability and stimulation of enzymes involved in lipolysis under pathological conditions, leading to disruption of membrane structure [15]. Along with changes in the behaviour of membrane-bound enzymes, receptors, and ion channels, this causes malfunction of the nervous system and cognitive disorders. The results are closely linked to the activation of inflammatory cytokine-releasing microglia and astrocytes (TNF-a, IL-1β, and IL-6). These spread and worsen neuroinflammation through a variety of mechanisms, including further PLA2 upregulation, platelet-activating factor generation, and nitric oxide synthase stimulation.
In the present study we screened important flavanoids of *Glycyrrhiza glabra*in-silico and based on their interaction capacity with cPLA2 and sPLA2, we have shown that Jaranol, Glycyrrhiza isoflavone, and Isoliquiritigenin are potent inhibitors for cPLA2 and sPLA2.

MATERIAL AND METHODS

Macrophage culture

Cell culture and treatment RAW264.7 macrophages were grown in DMEM supplemented with 10% heat-inactivated FBS and 1.0% penicillin-streptomycin solution in a humidified incubator with 5.0% CO2 at 37^{o}C.

cPLA2 activity assay and sPLA2 activity

cPLA2 activity was measured with a cPLA2 Assay Kit (Cayman Chemicals), as reported [16] using 1.5 mM arachidonoyl thio-PC as a synthetic substrate. Briefly, LPS stimulated/ macrophages homogenised in 3 mL of lysate buffer (10 mM Tris-HCl, pH 7.8, containing 1% Nonidet P-40, 0.15 M NaCl, and 1 mM EDTA), then chilled on ice for 30 min and centrifuged at 10 000 g for 15 min at 4C^{o}. cPLA2 activity was determined in the supernatant in the presence of the iPLA2 specific inhibitor bromoenol lactone (BEL, 10 lM) and of the sPLA2 specific inhibitor thioetheramide- PC (TE-PC, 50 lM), which were incubated with the samples for 15 min at 25 C^{o} prior to the assay. sPLA2 activity was measured with an sPLA2 assay kit (Cayman Chemicals), using the 1,2-dithio analog of diheptanoyl phosphatidylcholine, which serves as a substrate for most PLA2 enzymes, with the exception of cPLA2. Bee venom PLA2 was run as a positive control in the same assay to show a linear increase in the absorbance over the time range chosen. Absorbance was measured every minute after adding the substrate, for a total of 11 min. Activity was calculated for both cPLA2 and sPLA2 assays by measuring the absorbance at 414 nm, using the DTNB extinction coefficient of 10.66 per mM per cm, and reported as nmol/min/g of cytosolic protein).

Protein preparation

The required protein 1N29 and 1CJY were retrieved from the Protein databank website[17] and removed the pre-existed ligand molecules then cleaned the geometrical structure using freely accessible ArgusLab software [18]and MolProbityto add the missing atoms and correct the miss folded regions of each protein separately.

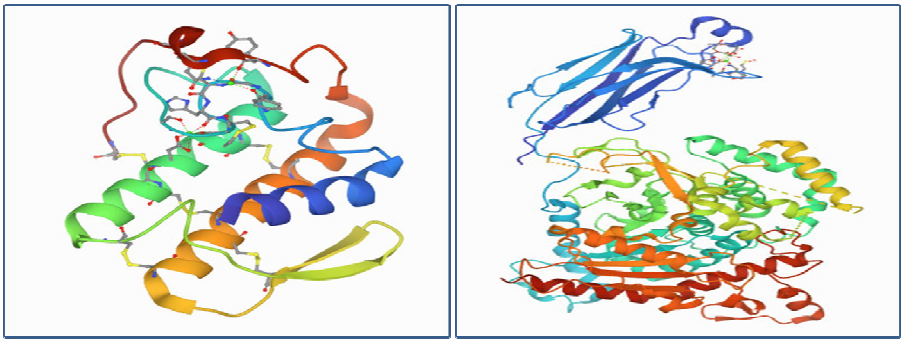

Fig.1. 3D structure of 1N29 and 1CJY proteins

Protein validation

The protein structures obtained were validated using the clashscore (Table 1) and Ramachandran Favoured regions (Fig.2). Further, the protein was taken for molecular docking procedure[19].

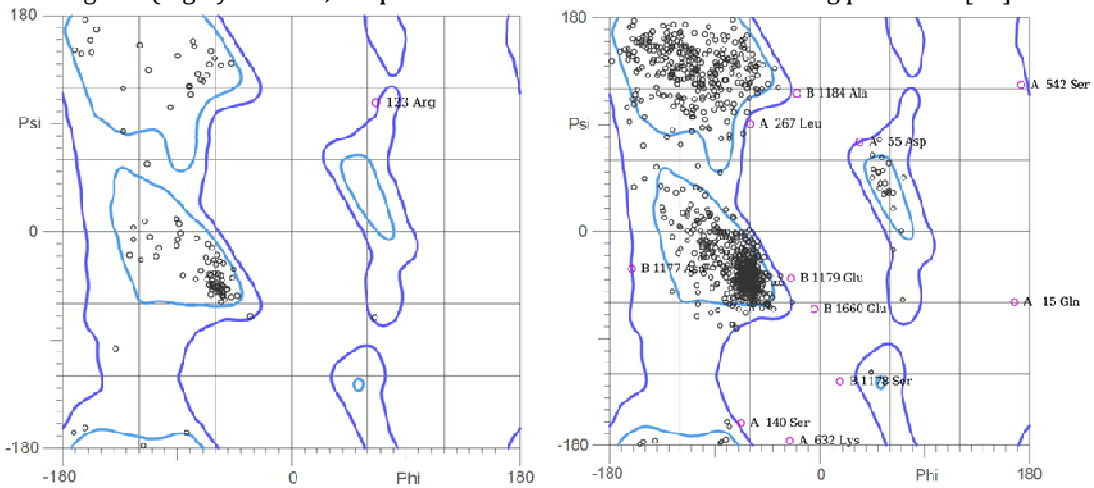

Fig.2: Structural analysis of 1CJY and 1N29 protein using Ramachandran plot, plotted using MolProbity.
(a) 92.6% (113/122) of all residues were in favored (98%) regions.
(b) 91.1% (1118/1227) of all residues were in favored (98%) regions.
98.3% (1206/1227) of all residues were in allowed (>99.8%) regions.
98.4% (120/122) of all residues were in allowed (>99.8%) regions.

Table 1: Clashscore structural analysis of proteins

Sl. No.	Target protein	Ramachandran Favoured region	Ramachandran outliers	Clashscore (Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms)
1	1N29	92.62%	1.64%	17.34
2	1CJY	91.12%	1.71%	18.55

Ligand preparation and validation

Ligands, Isoliquiritin, Liquiritin and Formononetin were downloaded from the PubChem database in sdf format since the molecular docking requires ligands in pdb format, downloaded drugs were converted to pdb format using open accessible tool called Open Bable GUI[20]. The geometry cleaning was done by the ArgusLab software for converted pdb files to achieve the proper 3D structure and orientation.

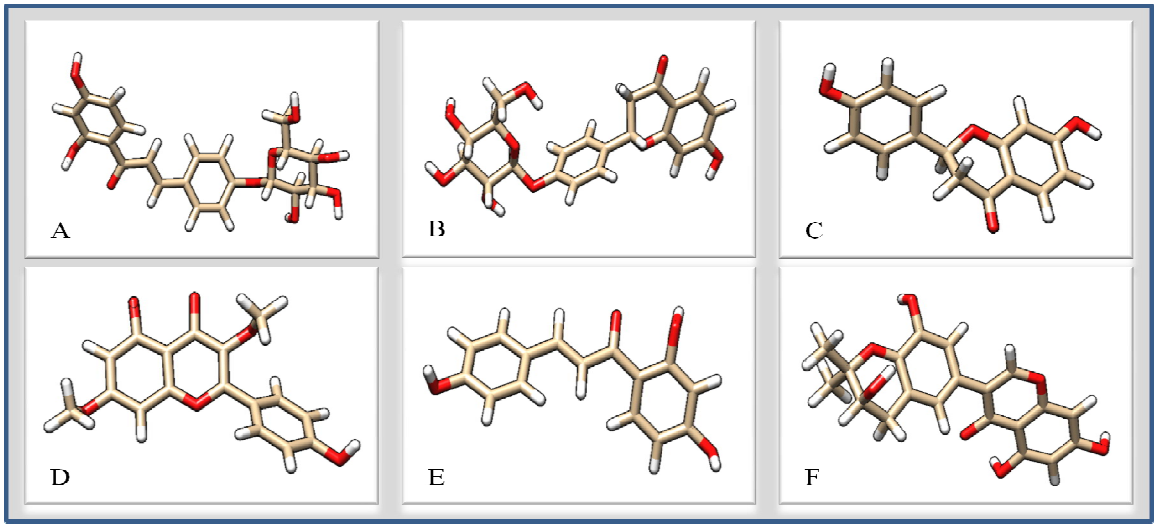

Fig.3.3D structure of ligands A) Isoliquiritin, B) Liqueitin, C) Formononetin D) Jaranol, E) Isoliquiritigenin and F) Glycyrrhizaisoflavone A.

Molecular Docking (MD)

Molecular docking interaction studies were done between the selected proteins and ligands to check if they can interact with each other forming bonded and non-bonded interactions. MD studies for the screened proteins and ligands were successfully performed using PyRx software [21].

RESULTS

Molecular docking:

The molecular docking results obtained by PyRx were observed for the intermolecular interactions using Discovery studio visualizer software. The docked proteins and ligands have achieved the significant result and parameters of acceptable counts. Based on docking and molecular interaction, binding energy, distance between the target proteins 1CJY, 1N29 and selected ligands, it can be inferred that the ligands are capable to exhibit modulatory functions in-silico. The flavonoidsGlycyrin, Isoliquiritin, liquiritin, formnonetien, Jaranol, Glycyrrhizaisoflavone A andIsoliquiritigenin were subjected to molecular docking with the target proteins 1N29 and 1CJY. All the molecules interact with sPLA2 and cPLA2 proteins with different bonds. Glycyrrhiza isoflavone A (Fig.3) and Jaranol (Fig.5) interacted with sPLA2 more efficiently with a binding affinity of 6.5 (Table 2).

Intermolecular interactions of 1N29 and selected compounds

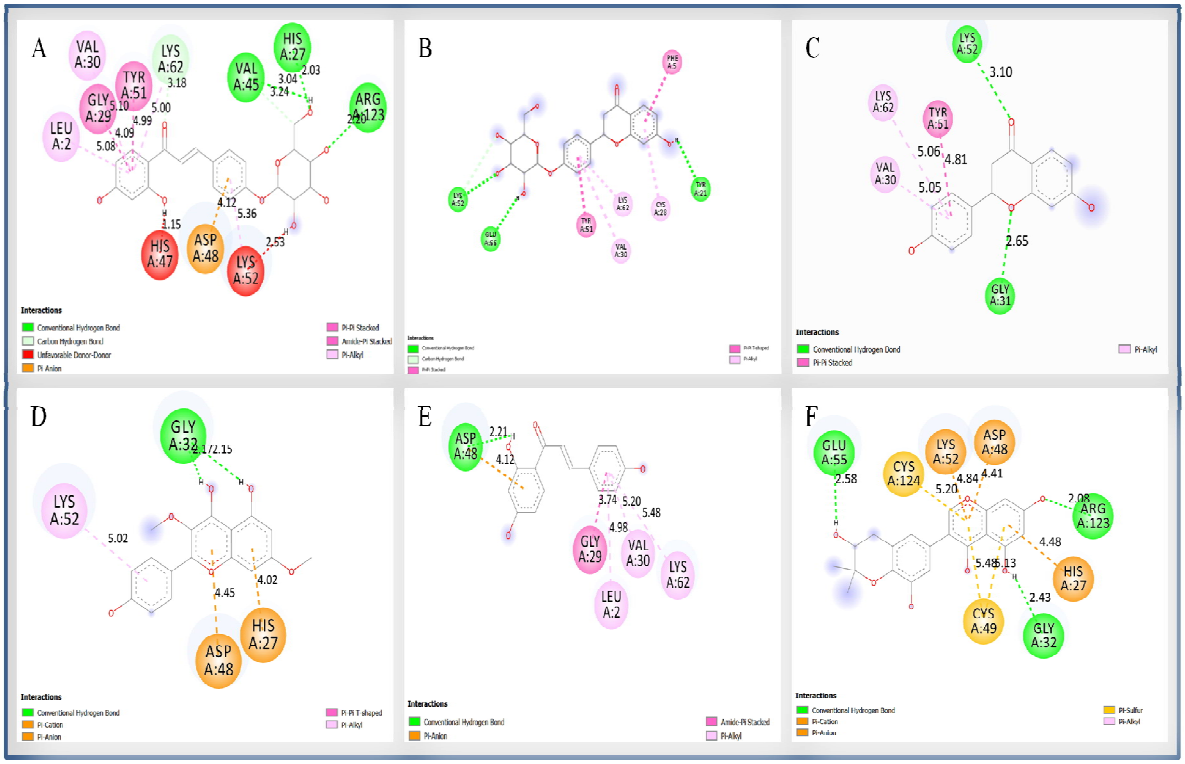

Fig.4:Intermolecular interaction of 1N29 and (a) Isoliquiritin (b)Liquritin (c) Formononetin (d) Jaranol (e) Isoliquiritigenin and (f) Glycyrrhizaisoflavone A.

Intermolecular interactions of 1CJY and selected compounds

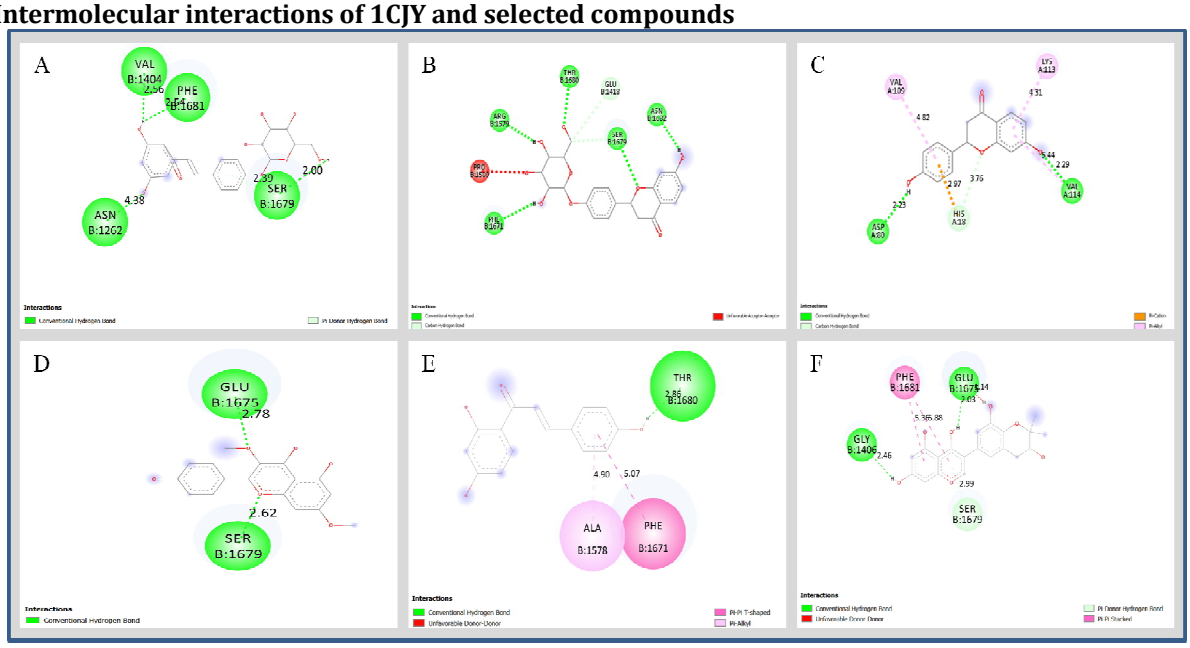

Fig.5: Intermolecular interaction of 1CJY and (a) Isoliquiritin (b) Liquritin (c) Formononetin (d) Jaranol (e) Isoliquiritigenin and (f) Glycyrrhizaisoflavone A.

Table 2: Molecular docking results the selected proteins with ligands

Target	Ligands	Residues	Binding Affinity	Interaction Distance
1N29 (sPLA2)	Glycyrin	GLY32 ASP48	-7	2.23 Å 3.22Å
	Glycyrrhizaisoflavone A	GLU55 GLY32 ARG123	-6.5	2.58 Å 2.08 Å 2.43 Å
	Isoliquiritigenin	ASP48	-7.5	2.21 Å
	Isoliquiritin	VAL45 LYS62 HIS27 ARG123	-8.7	3.24 Å 3.18 Å 2.03 Å 2.20 Å
	Jaranol	GLY32	-6.5	2.17 Å,2.15 Å
	Liquiritin	LYS 52 GLU55 LYS 52 CYS28 VAL30	-7.8	3.32 Å 2.44 Å 4.71 Å 5.10 Å 5.21 Å
	Formononetin	GLY32 VAL 30 LYS62 TYR51	-7.1	2.99 Å 2.65 Å 5.07 Å 480 Å
1CJY (cPLA2)	Isoliquiritigenin	THR1680	-5.9	2.86 Å
	Isoliquiritin	ASN1262 VAL1404 PHE1681 SER1679	-8.2	4.38 Å 2.56 Å 2.54 Å 2.00 Å
	Jaranol	GLU1675 SER1679	-6.2	2.78 Å 2.62Å
	Liquiritin	PHE167 ARG 1579 PRO 1580 GLU 1418 THR 1680 SER 1679 ASN 1682	-7.2	2.79 Å 2.13 Å 2.89 Å 3.53 Å 2.37 Å 1.89 Å
	Formononetin	LYS 113 VAL114 HIS18 ASP80	-7.1	2.39 Å 2.53 Å 3.81 Å 2.58 Å

cPLA2 and sPLA2 activity in LPS stimulated Macrophages

Pro-inflammatory enzymes and cytokines are increasingly attractive as therapeutic targets for a variety of inflammatory diseases and the inflammatory component of neurodegenerative disorders. The 14–18 kD secreted phospholipase A2s (sPLA2s) are of interest in this regard because of their accessibility in the circulation and because the local and systemic elevation of sPLA2s is associated with most forms of inflammation. The secreted isoforms are part of a growing family of PLA2 enzymes whose activity leads to the production of several potent mediators of inflammation. In the present investigation, LPS stimulated macrophages cPLA2 and sPLA2 activity was assessed in the presence and absence of flavonoids Jarnol, Glycyrrhizaisoflavone, Isoliquiritigenin. All these flavonoids inhibited cPLA2 and sPLA2 in stimulated macrophages. Jaranol, Glycyrrhizaisoflavone inhibited sPLA2 in a concentration-dependent manner (Fig.6). Pro-inflammatory enzymes and cytokines are increasingly attractive as therapeutic targets for a variety of inflammatory diseases and for the inflammatory component of neurodegenerative disorders. The 14–18 kD secreted phospholipase A2s (sPLA2s) are of interest in this regard because of their accessibility in the circulation and because local and systemic elevation of sPLA2s are associated with most forms of inflammation [1-5]. The secreted isoforms are part of a growing family of PLA2 enzymes whose activity leads to the production of several potent mediators of inflammation. In the present investigation LPS stimulated macrophages cPLA2 and sPLA2 activity was assessed in presence and absence of flavonoids Jaranol, Glycyrrhizaisoflavone and Isoliquiritigenin. All these flavonoids inhibited cPLA2 and sPLA2 in stimulated macrophages. Jaranol, Glycyrrhizaisoflavone inhibited sPLA2 in concentration dependent manner (Fig.6).Jaranol and Isoliquiritigenin inhibited cPLA2 activity is concentration dependent manner (Fig.7). Both the phosphplipases were found to be inhibited almost in a similar manner.

More recent experimental studies suggest sPLA2s are involved in traumatic and autoimmune precipitated neurodegeneration, and thus these enzymes are also a potential target for treatment of nervous system disorders. [6,7,33].

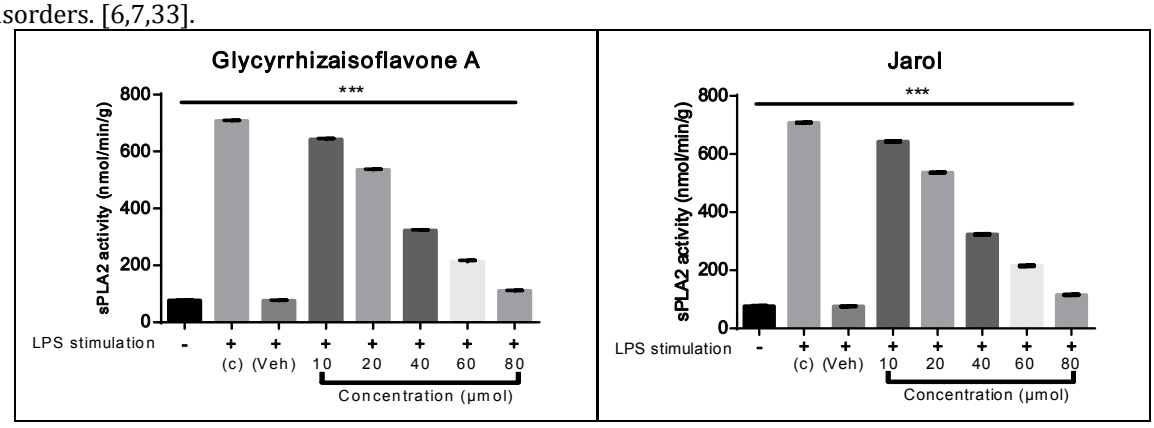

Fig.6: sPLA2 Activity in presence and absence of Glycyrrhiza isoflavone A and Jarnol. sPLA2 activity was measured using diheptanoylthio-PC as a synthetic substrate. Results are means ± SEM of 10–14 in dependent samples, each assayed in triplicate *p < 0.005

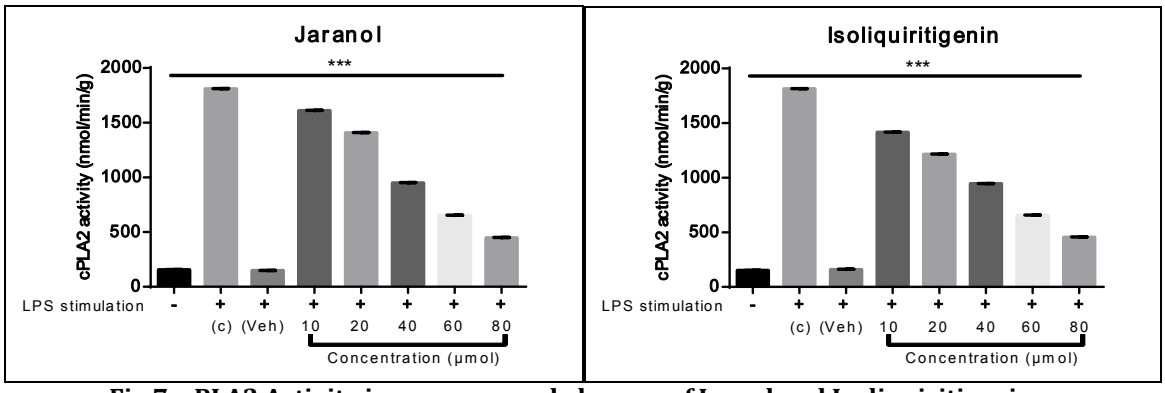

Fig.7: cPLA2 Activity in presence and absence of Jarnol and Isoliquiritigenin: cPLA2 activity was measured using arachidonoyl thio-PC as a synthetic substrate with a cPLA2 assay kit. The contributions of cPLA2 and sPLA2 were inhibited by pre-incubating the samples with 10 lM BEL and 50 lM TE-PC.

DISCUSSION

Activation of astrocytes or microglia by certain inflammatory mediators lead to neuroinflammation in central nervous system (CNS) which is associated with disorders, including brain infections, inflammation, ischemia, trauma, stroke and degenerative diseases, such as Alzheimer disease (AD), Parkinson's disease (PD) and axonal degeneration in multiple sclerosis (MS) [22]. The process of inflammation is generally associated with upregulation of cytokines, chemokines, reactive nitrogen species [23, 24] and prostaglandins (PGs) [25]. These prostaglandins along with leukotrienes act as pro-inflammatory lipid mediators which are produced due to the action of secretory phospholipase A_2-IIA ($sPLA_2$-IIA) which is known as a mediator to hydrolyze fatty acids from the ***sn***-2 position of membrane phospholipids, resulting in the generation of free arachidonic acid (AA) and lysophospholipids [26]. $sPLA_2$ [27, 28], originally isolated from inflammatory fluids and cells are identified to play a prominent role in inflammation and infectious diseases [29, 30]. $sPLA_2$ is generally localized in the hippocampus and cerebral cortex in brains of ischemia and AD [31, 32]. Interestingly, $sPLA_2$ interacts with cytosolic phospholipase A_2 ($cPLA_2$) due to its phospholipase enzymatic activity, to supply AA for other biosynthetic pathways [33].

Rosmarinic acid, tetrahydrocurcumin, dihydrocurcumin and hexahydrocurcumin showed better binding than curcumin [34], synthetic Pyrazolo[3,4-d] pyrimidines derivatives also were shown to have sPLA2 binding efficacy [35]. The naturally occurring phenolic compound derivatives such as rosmarinic acid 3'-O-beta-glucoside, 4-nerolidylcatechol, rosmarinic acid methyl ester, quercetin 3-O-malonylglucoside, quercetin pentaacetate, and rosmarinic acid have shown better interaction with sPLA2. The plant-derived phenolic compound such as catechin forms strong interactions with PLA2 [36], betulinic acid, a constituent of Coryphataliera (Roxb.) exhibited better interaction and pharmacokinetic properties [37], bromelain-phytochemical complex inhibitors via a combination of in silico and in vitro methods. Bromelain-amenthoflavone displays antagonistic effects on Pla2. Bromelian-asiaticoside and bromelain-diosgenin displayed synergistic effects with sPLA2 [38].

Similar effects have shown by the bilobetin and ginkgetin can inhibit the sPLA2 and subsequently inhibit the production of TNF alpha and iNOs[39]. The compound SB203347 and Scalaradial have reported being potent sPLA2 inhibitors [40].Jaranol and Isoliquiritigenin inhibited cPLA2 activity is a concentration-dependent manner. Both inhibit almost a similar manner. Several investigators have demonstrated cPLA2 inhibitors, i e ASB14780 potent cPLA2 inhibitors [41]. The thiazolyl ketones as inhibitors of $cPLA_2$ and demonstrated the in vivo anti-inflammatory activity of inhibitor GK470[42], pyrrophenone[43] The potent cPLA2 inhibitors 2-Oxoester have exhibited the potent cPLA2 inhibitory activity [44]. Apart from this ginko biloba extract has been shown to inhibit the cPLA2 activity [45].The phenolic content found more in the medicinal plant known to have a beneficial effect on inflammation by blocking the cPLA2 [46]. The arachidonic acid (AA) analogs also shown to inhibit the cPLA2 [47], and capable of reducing reactive oxygen species (ROS) and nitric oxide (NO) production in stimulated microglial cells. Some biomolecules quinacrine and chloroquine, arachidonyl trifluoromethyl ketone, bromoenol lactone, cytidine 5-diphosphoamines, and vitamin E, inhibit phospholipase A2 activity [48], cubebin [49],Benzyl 4-nitrobenzenecarbodithioate[50].

Conclusion

Cytosolic phospholipase A2 (cPLA2) and secreted phospholipase A2 (sPLA2) are well known to promote inflammatory metabolic diseases such as atherosclerosis, hyperlipidemia, obesity, etc., throughgeneration of active lipid metabolites. Inhibiting the activity of cPLA2 and sPLA2 might be very important to control inflammatory reactions. We selected *Glycyrrhiza glabra* which is known to be usedin foods and medicinal remedies for thousands of years. The plant contains a number of flavanoids which carry great potential to treat diseases. Flavanoids which could interact with cPLAs and sPLA2 were screened through in-silico approach. Jaranol, Glycyrrhizaisoflavone A and Isoliquiritigenin were found to interact with higher affinity when compared to other flavanoids. Observing the molecular docking and molecular interaction, binding energy, and distance between the target proteins 1N29, 1CJY and selected ligands, it is found that the ligands are capable to exhibit the modulatory functions. Further, these flavanoids were tested in vitro on cells stimulated with LPS and the results revealed that these flavanoids inhibited the activity of cPLA2 and sPLA2 on a concentration dependent manner.

REFERENCES

1. Burke JE, Dennis EA. (2009). Phospholipase A2 biochemistry. Cardiovasc Drugs Ther. 23:49–59.
2. Murakami M, Taketomi Y, Miki Y, Sato H, Hirabayashi T, Yamamoto K. (2011). Recent progress in phospholipase A research: from cells to animals to humans. Prog Lipid Res. 50:152–92.

3. Leslie CC. (2004). Regulation of arachidonic acid availability for eicosanoid production. Biochem Cell Biol. 82:1–17.
4. Samuelsson B, Dhalen SE, Lindgren JA, Rouzer CA, Serhan CN. (1987). Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science. 237:1171–6.
5. Niknami M, Patel M, (2009). Witting PK, Dong Q. Molecules in focus: cytosolic phospholipase A2-alpha. Int J Biochem Cell Biol. 41:994–7.
6. Cunningham TJ, Souayah N, Jameson B, Mitchell J, Yao L: (2004). Systemic treatment of cerebral cortex lesions in rats with a new secreted phospholipase A2 inhibitor. J Neurotrauma. 21:1683-1691.
7. Murakami M, Kudo I. (2002). Phospholipase A2. J Biochem. 131: 285–92.
8. Kramer RM, Hession C, Johansen B, Hayes G, McGray P, et al. (264).Structure and properties of a human non-pancreatic phospholipase A2. J Biol Chem.1989; 5768–5775.
9. Seilhamer JJ, Plant S, Pruzanski W, Schilling J, Stefanski E, et al. (1989). Multiple forms of phospholipase A2 in arthritic synovial fluid. J Biochem. 106: 38–42.
10. Jaulmes A, Janvier B, Andreani M, Raymondjean M. (2005). Autocrine and paracrine transcriptional regulation of type IIA secretory phospholipase A2 gene in vascular smooth muscle cells. ArteriosclerThrombVasc Biol. 25: 1161–1167.
11. Ibeas E, Fuentes L, Martin R, Hernandez M, Nieto ML. (2009). Secreted phospholipase A2 type IIA as a mediator connecting innate and adaptive immunity: new role in atherosclerosis. Cardiovasc Res. 81: 54–63.
12. Chalbot S, Zetterberg H, Blennow K, Fladby T, Grundke-Iqbal I, et al. (2009). Cerebrospinal fluid secretory Ca2+-dependent phospholipase A2 activity is increased in Alzheimer disease. Clin Chem. 55: 2171–2179.
13. Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, et al. (2002). Human group IIA secretory phospholipase A2 induces neuronal cell death via apoptosis. Mol Pharmacol. 61: 114–126.
14. Murakami M, Kambe T, Shimbara S, Kudo I. (1999). Functional coupling between various phospholipase A2s and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. J Biol Chem. 274: 3103–3115.
15. Cunningham TJ, Yao L, Oetinger M, Cort L, Blankenhorn EP, (2006). Greenstein JI: Secreted phospholipase A2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroinflammation; 3:26.
16. Bosetti Francesca and Gayani R. Weerasinghe. (2003). The expression of brain cyclooxygenase-2 is down-regulated in the cytosolic phospholipase A2 knockout mouse. Journal of Neurochemistry.87(6):1471-1477.
17. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., &Bourne, P. E.(2000). The Protein Data Bank. Nucleic acids research. 28(1), 235–242.
18. Naz, A., Bano, K., & Akhtar, N. (2009). Conformational analysis (geometry optimization) of nucleosidic antitumor antibiotic showdomycin by Arguslab 4 software. *Pakistan journal of Pharmaceutical Sciences.* 22(1): 78-82.
19. Wang, W., Xia, M., Chen, J., Deng, F., Yuan, R., Zhang, X., & Shen, F. (2016). Data set for phylogenetic tree and RAMPAGE Ramachandran plot analysis of SODs in Gossypium raimondii and G. arboreum. Data in brief. 9: 345–348.
20. O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. J Cheminform. 3: 33.
21. Dallakyan, S., & Olson, A. J. (2006). Small-molecule library screening by docking with PyRx. Methods Mol Biol. 1263; 243-250.
22. Polazzi E, Contestabile A. (2002). Reciprocal interactions between microglia and neurons: from survival to neuropathology. Rev Neurosci. 13: 221–242.
23. Kummer MP, Hermes M, Delekarte A, Hammerschmidt T, Kumar S, et al. (2011). Nitration of tyrosine 10 critically enhances amyloid beta aggregation and plaque formation. Neuron. 71: 833–844.
24. McGeer EG, McGeer PL.(2003). Inflammatory processes in Alzheimer's disease. Prog NeuropsychopharmacolBiol Psychiatry. 27: 741–749.
25. Guo JT, Yu J, Grass D, de Beer FC, Kindy MS. (2002). Inflammation-dependent cerebral deposition of serum amyloid a protein in a mouse model of amyloidosis. J Neurosci. 22: 5900–5909.
26. Carasso RL, Vardi J, Rabay JM, Zor U, Streifler M. (1977). Measurement of prostaglandin E2 in cerebrospinal fluid in patients suffering from stroke. J NeurolNeurosurg Psychiatry. 40: 967–969.
27. Gao W, Schmidtko A, Wobst I, Lu R, Angioni C, et al. (2009). Prostaglandin D2 produced by hematopoietic prostaglandin D synthase contributes to LPS-induced fever. J PhysiolPharmacol. 60: 145–150.
28. Murakami M, Kudo I. (2002). Phospholipase A2. J Biochem. 131: 285–292.
29. Kramer RM, Hession C, Johansen B, Hayes G, McGray P, et al. (1989). Structure and properties of a human non-pancreatic phospholipase A2. J Biol Chem. 264: 5768–5775.
30. Seilhamer JJ, Plant S, Pruzanski W, Schilling J, Stefanski E, et al. (1989). Multiple forms of phospholipase A2 in arthritic synovial fluid. J Biochem.106: 38–42.
31. Jaulmes A, Janvier B, Andreani M, Raymondjean M.(2005). Autocrine and paracrine transcriptional regulation of type IIA secretory phospholipase A2 gene in vascular smooth muscle cells. ArteriosclerThrombVasc Biol. 25: 1161–1167.
32. Ibeas E, Fuentes L, Martin R, Hernandez M, Nieto ML. (2009). Secreted phospholipase A2 type IIA as a mediator connecting innate and adaptive immunity: new role in atherosclerosis. Cardiovasc Res. 81: 54–63.
33. Chalbot S, Zetterberg H, Blennow K, Fladby T, Grundke-Iqbal I, et al. (2009). Cerebrospinal fluid secretory Ca2+-dependent phospholipase A2 activity is increased in Alzheimer disease. Clin Chem. 55: 2171–2179.

34. Dileep, K.V., Tintu, I. &Sadasivan, C. (2011). Molecular docking studies of curcumin analogs with phospholipase A2. Interdiscip Sci Comput Life Sci. 3: 189.
35. Yadava, U., Singh, M., &Roychoudhury, M. (2013). Pyrazolo[3,4-d]pyrimidines as inhibitor of anti-coagulation and inflammation activities of phospholipase A 2 : insight from molecular docking studies. Journal of biological physics. 39 (3): 419–438.
36. Ramesh kumara D, P. Seetha Lakshmi, N. Saravanic and Srinivasan Marimuthu. (2012). In silico Molecular Docking Studies on Porcine Pancreatic Phospholipase A2 against Plant extracts of Phenolic Inhibitors. International Journal of Research in Biomedicine and Biotechnology. 2(3): 8-16.
37. Khan, M.F., Nahar, N., Rashid, R.B. et al. (2018). Computational investigations of physicochemical, pharmacokinetic, toxicological properties and molecular docking of betulinic acid, a constituent of Coryphataliera (Roxb.) with Phospholipase A2 (PLA2). BMC Complement Altern Med. 18: 48.
38. Mohamed Tap, F.; Abd Majid, F.A.; Ismail, H.F.; Wong, T.S.; Shameli, K.; Miyake, M.; Ahmad Khairudin, N.B. (2018). In Silico and In Vitro Study of the Bromelain Phytochemical Complex Inhibition of Phospholipase A2 (Pla2). Molecules: 23: 73.
39. Baek SH, Yun SS, Kwon TK, Kim JR, Chang HW, Kwak JY, Kim JH, Kwun KB.(1999). The effects of two new antagonists of secretory PLA2 on TNF, iNOS, and COX-2 expression in activated macrophages. Shock. ;12(6):473-8.
40. Marshall, L. A., R. H. Hall, J. D. Winkler, A. Badger, B. Bolognese, A. Roshak, P. L. Flamberg, C. M. Sung, M. Chabot-Fletcher, and J. L. Adams. (1995)."SB 203347, an inhibitor of 14 kDa phospholipase A2, alters human neutrophil arachidonic acid release and metabolism and prolongs survival in murine endotoxin shock." Journal of Pharmacology and Experimental Therapeutics.;274(3): 1254-1262.
41. Tomoo, T.; Nakatsuka, T.; Katayama, T.; Hayashi, Y.; Fujieda, Y.; Terakawa, M.; Nagahira, K. (2014). Design, synthesis, and biological evaluation of 3-(1-aryl-1H-indol-5-yl)propanoic acids as new indole-based cytosolic phospholipase $A_2\alpha$ inhibitors. J. Med. Chem. 57: 7244– 7262,
42. Kokotos, G.; Feuerherm, A. J.; Barbayianni, E.; Shah, I.; Sæther, M.; Magrioti, V.; Nguyen, T.; Constantinou-Kokotou, V.; Dennis, E. A.; Johansen, B.(2014). Inhibition of group IVA cytosolic phospholipase A_2 by thiazolyl ketones in vitro, ex vivo, and in vivo. J. Med. Chem. 57: 7523–7535.
43. Li, Z.; Qu, M.; Sun, Y.; Wan, H.; Chai, F.; Liu, L.; Zhang, P. (2018). Blockage of cytosolic phospholipase A_2 alpha sensitizes aggressive breast cancer to doxorubicin through suppressing ERK and mTOR kinases. Biochem. Biophys. Res. Commun. 496:153-158,
44. Anastasia Psarra, Maroula G. Kokotou, GerasimiaGaliatsatou, Varnavas D. Mouchlis, Edward A. Dennis, and George Kokotos, (2018). Highly Potent 2-Oxoester Inhibitors of Cytosolic Phospholipase A2 (GIVA cPLA2), ACS Omega. 3 (8): 8843-8853.
45. Gargouri B, Carstensen J, Bhatia HS, Huell M, Dietz GPH, Fiebich BL.(2018). Anti-neuroinflammatory effects of *Ginkgo biloba* extract EGb761 in LPS-activated primary microglial cells. Phytomedicine. 15(44):45-55.
46. Chuang DY, Simonyi A, Cui J, Lubahn DB, Gu Z, Sun GY. (2016). Botanical Polyphenols Mitigate Microglial Activation and Microglia-Induced Neurotoxicity: Role of Cytosolic Phospholipase A2. Neuromolecular Med. ;18(3):415-25.
47. Ng CY, Kannan S, Chen YJ, Tan FCK, Ong WY, Go ML, Verma CS, Low CM, Lam Y. (2017). A New Generation of Arachidonic Acid Analogues as Potential Neurological Agent Targeting Cytosolic Phospholipase A_2. Sci Rep. ;7(1):13683.
48. Farooqui AA, Ong WY, Horrocks LA. (2006). Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. Pharmacol Rev. 58(3):591-620.
49. Muhammad Helmi Nadri, Wan MohdNuzul Hakimi Wan Salleh. (2020). Molecular Docking Studies of Phytochemicals from Piper Species as Potential Dual Inhibitor of Group X Secreted Phospholipase A2 (SPLA2-X) and Cyclooxygenase-2 (COX-2). Research J. Pharm. and Tech. 13(5): 2181-2186.
50. Isabel HenaoCastañeda, Jaime Andrés Pereañez, Lina María Preciado and Jorge Jios. (2020). Sulfur Compounds as Inhibitors of Enzymatic Activity of a Snake Venom Phospholipase A2: Benzyl 4-nitrobenzenecarbodithioate as a Case of Study, Molecules. 25: 1373.

CITATION OF THIS ARTICLE

Avinash KO, Sushma Pradeep, Chandan Shivamallu, Gopenath TS, Kiran Kumar MN, Kanthesh BM, JR Kumar. *In Silico* Screened Flavanoids of *Glycyrrhiza glabra* Inhibit CPLA2 And SPLA2 In Lps Stimulated Macrophages. Bull. Env. Pharmacol. Life Sci., Vol 10[5] April 2021 : 72-80.