Interaction between C-Reactive Protein and Phytochemical(s) from *Calotropis procera*: An Approach on Molecular Docking

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**Keywords**: Virtual screening; Molecular docking; In silico study; *Calotropis procera*; Phytochemicals; Anti-inflammation

**Abstract.** The present study was attempted to detect potential phytoconstituents in *C. procera* against inflammation and pain. CRP is known to be increased up to 10,000 fold when acute inflammation take place in human. The interaction between C-reactive protein and phytochemical(s) from *Calotropis procera* was carried out with the help of molecular docking by using PyRx software (Ver. 0.8) and LigPlot software (Ver. 1.4) to compare energy value and binding site of phytochemicals in reference to established synthetic non-steroidal anti-inflammatory drugs (NSAIDs). The data suggest that the interaction between CRP and two phytochemicals namely methyl myrisate (-3.0) and methyl benenate (-3.2) showed close energy value (kcal/mol) and binding site in comparison to paracetamol (-3.9), ibobufen (-4.2) while three phytochemicals viz. β-sitosterol (-5.6), uzarigenin (-5.5) and anthocyanins (-5.4) closely related to indomethacin (-5.2) in relation to energy value and binding site. In conclusion, based on molecular docking we found few phytochemicals of *C. procera* that can be used as lead compound(s) in future drug development as analgesic and anti-inflammatory agent at low cost. It is also suggested to carry out functional assay of predicted compounds to validate suitability of this lead.

1. Introduction

C-reactive protein or CRP belongs in the pentraxin family having calcium ion dependent plasma protein [1]. The family is named after viewing under electron microscope that radial symmetry having five monomers, emerging from the Greek word, penta (five) ragos (berries), is highly conserved in evolution [2]. The human CRP molecule is composed of five identical non-glycosylated polypeptide subunits; individual subunit is contained 206 amino acid residues. The protomers are non-covalently attached in an annular configuration with cyclic pentameric symmetry. According to Tillet and Francis [3], CRP has been investigated in Oswald Avery’s laboratory during the studies with *Streptococcus pneumoniae* infection in human subjects.

Generally, CRP is found in plasma and its circulating concentration is increased majorly in a cytokine-mediated response during tissue injury, infection and inflammation. In addition, serum CRP values are estimated in clinical study to identify disease progression [4-6]. This human CRP protein is homologous mainly to chordates and also other invertebrates’ CRP, which increased during inflammation through systemic circulation [7]. CRP is the potent inflammatory markers and is an important area to carry out detail research worldwide. Unlike other markers of inflammation, CRP levels can easily be estimated at a low cost with proper sensitivity.

The inflammatory process is always attributed by the secretion of various mediators such as prostaglandins, histamine, bradykinin, leukotrienes, platelet activating factor (PAF) and production of chemicals from tissues and migrating cells [8-10]. On the other hand, inflammation is a pathophysiological response of living tissue after injuries that lead to local accumulation of plasma fluid and blood cells.
It was reported that tissue inflammation leads to increased level of CRP. There are several mediators of inflammatory processes, CRP is one of the protein that showed pleiotropic effects [11]. According to literatures both pro-inflammatory (cytokines mediated induction of diseases e.g. inflammation) and anti-inflammatory (reduction of inflammation and recovery) activities have been documented [7,12-14]. In addition, study of in vivo anti-inflammatory effects, CRP has been observed to increase the expression of interleukin-1 (IL-1) receptor antagonist [15] and increased the secretion of the anti-inflammatory cytokine interleukin-10 [16-17] but inhibited the synthesis of interferon-γ [17]. However, many other functions that can be regarded as pro-inflammatory. CRP initiates and increases the mechanism of phagocytosis, regulates the expression of adhesion molecules present in endothelial cells, inhibits the nitric-oxide synthase enzyme expression in the endothelial cells of aorta [18], stimulates IL-8 secretion from several cell types, induces the expression and function of plasminogen activator inhibitor-1 and also enhances the availability of IL-1, IL-6, IL-18, and tumour necrosis factor-α [19]. In other experiments of in vitro, it was observed that properties are compatible with the net in vivo effects of CRP in mice, it is likely that the function of CRP is depended upon tissue microenvironment and intense inflammatory responses depending on the microenvironment [7].

In virtual screening, proteins (receptors) are the main molecular targets to detect drug action easily. Several compounds (ligands) either synthetic drugs or phytochemicals, bind to the protein targets to show the favourable or inhibitory effects, which help in new and efficient drug development as a lead molecule. The virtual screening reveals large libraries of drug-like compounds, which are commercially available, computationally screened against targets of recognized structure, and those that are predicted to bind properly in an experiment [20-22]. However, database screenings do not depend on the molecules that are structurally novel as these molecules have already been previously synthesized by chemical/drug producers. In de novo drug design, the three-dimensional structure of the receptor is utilized specially to design structurally new molecules that have not been synthesized prior to using ligand-developing programs and the intuition of the medicinal chemist [23]. Therefore, in silico predictions play an important role in the drug design and discovery process within the periphery of pharmaceutical research.

From ancient time medicines from different plants have been in practice traditionally. Among Indian medicinal plants, C. procera (Aiton) R. Br., belongs to milkweed family, used traditionally for various diseases [24-25]. This plant and its different parts have already been investigated for potent medicinal values towards prevention of several diseases [25-32]. Besides various diseases, inflammatory disease is one of the major health concern. It leads to internal and external inflammation in tissues, organs etc. There are well established anti-inflammatory synthetic drugs, which help as pain killer as well as recovery of swelling in tissues however, may cause side effect like ulcer in gastrointestinal tract [33-37]. Moreover, aqueous flower extract of C. procera has been documented to arrest pain, fever and inflammation [38-39] while ethanolic extract of root bark showed anti-inflammatory properties without any side effect [9,40].

The objective of the present study is to know binding affinity and energy of CRP towards different phytochemicals present in C. procera in reference to established common NSAIDs through molecular docking. The comparative analysis between these phytochemicals and known synthetic drugs with the CRP have been probed by using computational prediction for new drug design for inflammatory disorders.

2. Materials and Methods

2.1 Selection of protein

The structural information C-reactive protein or CRP (receptor) of human was obtained from European Protein Data Bank (http://www.ebi.ac.uk/pdbe). The crystal structure of CRP complexed with five phosphocholine molecules and calcium-complexed (ID: 1b09) was selected according to the wwPDB validation report [5]. The file was procured as .ent extension, which was manually saved to .pdbqt format for docking by using PyRx Virtual Screening Tool developed by The
Prior to use PyRx software, the water molecules were deleted and the polar hydrogens were added by using AutoDock tool. The crystal structure of CRP is depicted in Fig. 1.

**Figure 1.** Cartoon representation of the crystal structure of CRP. The five subunits are shown in different colours. Calcium complexes and phosphocholines are shown in atomic sphere and stick and ball representation. The central cavity of the CRP is visible in this orientation.

### 2.2 Selection of compounds

Three compounds as common anti-inflammatory drugs viz. ibobrufen, indomethacin and paracetamol were selected from previous study [10]. There were 19 compounds viz. methyl myrisate, methyl behenate, anthocyanin, uzarigenin, lupeol, terpenol ester, calotropursenyl acetate, calopriedelenyl, β-amyrin, α-amyrin, calotropoleanyl ester, uscharin, calotropin, cardenolides, N-dotriacont-6-ene, glyceryl mono-oleoyl-2-phosphate, glyceryl-1, 2-dicapriate-3-phosphate, quercetin-3-rutinoside and β-sitosterol reported in literature as bioactive phytochemicals found in *C. procera* [32]. Among these 19 compounds, the detail information for only 12 compounds viz. methyl myrisate, methyl behenate, anthocyanins, uzarigenin, lupeol, calotropursenyl acetate, β-amyrin, α-amyrin, uscharin, calotropin, quercetin-3-rutinoside and β-sitosterol as well as 3 drugs viz. ibobrufen, indomethacin and paracetamol were obtained from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/compound). The three-dimensional (3D) structure of all the compounds both phytochemicals and synthetic drugs were obtained from CORINA online software (http://www.mol-net.de) after incorporating the Canonical SMILES string for each chemical that taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/compound) and are depicted in Table 1. In same software (http://www.mol-net.de), each compound was downloaded as .pdb file. The protein-ligand binding interaction was studied individually from the 12 above mentioned phytoconstituents viz. methyl myrisate, methyl behenate, anthocyanin, uzarigenin, lupeol, calotropursenyl acetate, β-amyrin, α-amyrin, uscharin, calotropin, quercetin-3-rutinoside and β-sitosterol as well as 3 drugs viz. ibobrufen, indomethacin and paracetamol. All the compounds (ligands) and CRP (receptor) were converted to .pdbqt format from .pdb file prior to molecular docking study.

### 2.3 Virtual screening

The virtual screening was done through PyRx software (Virtual Screening Tool, Ver 0.8) developed by Trott and Olson [41]. The molecular docking was visualized the output .pdbqt file by
using AutoDock Vina software, developed by Morris et al. [42] and the results of three-dimensional structure were rendered by using MGL Tools. The PyRx software is an easy virtual screening with minimum steps and time to obtain docking output file.

This software is combination of AutoDock Vina, AutoDock 4.2, Mayavi, Open Babel and Python tools. It is also non-commercial, less time consuming docking program that basically predict receptor-ligand interactions along with providing energy value for each test compound. Docking of 12 phytochemicals with CRP (PDB ID: 1b09) was analysed for the docking of phytoconstituents (ligands) and the CRP (receptor) to identify the residues involved in the study of receptor-ligand interactions. All the ligands and receptor file were individually taken prior converted to .pdbqt file format. The present software shows docking by obtaining energy value for individual phytoconstituents as well as synthetic drugs. Finally, all the 12 phytochemicals were analysed by comparing with previously established 3 synthetic NSAIDs to detect similarities on binding position and energy value. The resultant structural complexes of the individual phytochemical and with CRP were finally analysed by using the LigPlot software, Ver. 1.4 developed by Wallace et al. [43], to determine some specific contacts between the atoms of the test compounds and amino acids of the CRP.

3. Results and Discussion

The docking results clearly revealed that the interaction of the phytochemicals (ligands) present in the different parts of Calotropis procera with CRP (receptor), data were energetically favourable. It was observed from Table 1 that the energy values for two phytochemicals viz. methyl myrisate (-3.0) and methyl behenate (-3.2) were observed highest value while lowest values for another two phytochemicals namely uscharin (-6.7) and calotropin (-7.0), followed by α-amyrin (-6.6), β-amyrin (-6.5), lupeol (-6.4), quercetin-3-rutinoside (-6.3), calotropursenyl acetate (-6.2), β-sitosterol (-5.6), uzarigenin (-5.5) and anthocyanins (-5.4) in relation to established anti-inflammatory drugs viz. paracetamol (-3.9), ibobrufen (-4.2). However, indomethacin (-5.2) was lower than paracetamol and ibobrufen. All above-mentioned data were procured from PyRx software [41] and molecular surface representation along with binding position for each compound was observed through AutoDockTool interface [43] and is exhibited in Fig 2 (A-L) and 3 (A-C).

In Fig 2 (a-l), the docking results for individual phytochemical was observed for binding sites and energy value (kcal/mol) that few compounds viz. methyl myrisate and methyl behenate closely related to paracetamol and ibobrufen while β-sitosterol, uzarigenin and anthocyanins closely related to indomethacin and Figs. 2 (a-l) and 3 (a-c) have showed binding interactions with residues through schematic representation for each phytochemical and synthetic drug.

The compounds such as α-amyrin, β-amyrin, anthocyanins, calotropursenyl acetate, lupeol, methyl myrisate and methyl behenate while in case of drug indomethacin showed binding inside the central cavity of CRP and mainly hydrophobic in nature. The residues such as Val91, Arg116, Lys114, Val11, and Asp112 and Thr90 for α-amyrin, Lys114, Arg116, Val111 and Val86 for anthocyanins, Glu88, Thr90, Val111, Arg116, Lys114, Val91 and Ala92 for β-amyrin, Lys114, Asp112, Tyr175, Val111, Arg116, Glu88, Pro87 and Val86 for calotropursenyl acetate, Val86, Val11, Arg116, Glu88 and Pro87 for lupeol, Lys114, Val11, Val91, Glu88, Arg116, Val86 and Thr90 for methyl behenate and Lys114, Val111, Arg116, Val91, Val86 and Glu88 for methyl myrisate while Lys114, Val111, Arg116, Val86, Glu88, Pro87, Thr90 and Asp112 for indomethacin as residues of CRP were found to form hydrophobic contacts.

In case of other phytochemicals viz. β-sitosterol, quercetin-3-rutinoside and calotropin showed binding inside the central cavity with CRP same as synthetic drugs. It was found β-sitosterol, quercetin-3-rutinoside and calotropin have 1, 4 and 1 nos. while ibobrufen and paracetamol have 3 and 2 nos. respectively hydrogen bonds with CRP during interaction study. It was observed from each schematic two-dimensional diagram, the hydrogen bonds formation was involved with particular residues for each phytochemical like β-sitosterol: Thr90; quercetin-3-rutinoside: Val91, Glu88 and Arg116 and calotropin: Lys114 while for synthetic drugs such as ibobrufen: Thr90 and Glu88 and paracetamol: Val91 and Arg116 respectively. The hydrophobic interactions...
interactions play an important role in binding with CRP for rest natural and synthetic compounds. The residues were observed for hydrophobic interactions in each phytochemical as Glu88, Pro87, Val86, Val111, Arg116, Val91, Lys114 and Ala92 for β-sitosterol, Lys114, Asp112, Val111, Thr90 and Val86 for quercetin-3-rutinoside and Glu88, Val111, Val86, Asp112, Thr90, Val91 and Ala92 for calotropin while in each synthetic drug as Val89, Arg116, Val111 and Val86 for ibobrufen and Val111 and Val86 for paracetamol respectively.

It was reported that during acute phase of inflammation, many plasma proteins are increased severely including CRP, the protein for the present study. According to Black et al. [7], CRP level increases faster and in numbers as more than 1000 folds in human plasma due to an acute inflammatory condition while Shrivastava et al. [44] have documented CRP level increases up to 10000 folds during acute inflammation. Usually CRP gene is expressed by the liver during the inflammation [45]. Inflammation along with pain disorders in human is of serious concern and uncontrolled use of NSAIDs viz. ibobrufen, paracetamol, indomethacin etc. for the recovery of pain and inflammation, ultimately lead to severe side effects like ulcer in gastro-intestinal tract [35-37]. Interestingly, C. procera is one the plant that contains several phytochemicals and few of these have already been documented for anti-inflammatory properties [9,38-39], which supported the present computational prediction. The present virtual screening for binding of CRP (receptor) with phytochemicals (ligands) of C. procera have supported previous experimental study as anti-inflammatory activity [46-47] because CRP is a potent marker of inflammation [48]. Also this plant used as folk medicine, however, no one has attempted before the molecular docking approach as CRP along with phytochemicals of C. procera either individually and/or combinations can be developed in new drug designing for better therapeutic efficacies against inflammation and pain by particularly targeting CRP.

Table 1. Binding energies of the compounds from C. procera and anti-inflammatory drugs with CRP

| Sl. No. | Ligands       | Structure in 3D | Binding affinity (kcal/mol) |
|--------|---------------|-----------------|-----------------------------|
| 1.     | Methyl myrisate | ![Methyl myrisate](#) | -3.0                        |
| 2.     | Methyl behenate | ![Methyl behenate](#) | -3.2                        |
| 3.     | Anthocyanins   | ![Anthocyanins](#) | -5.4                        |
| 4.     | Uzarigenin     | ![Uzarigenin](#)  | -5.5                        |
| 5.     | β-sitosterol   | ![β-sitosterol](#) | -5.6                        |
|   |   |   |
|---|---|---|
| 6. | Calotropursenyl acetate | -6.2 |
| 7. | Quercetin-3-rutinoside | -6.3 |
| 8. | Lupeol | -6.4 |
| 9. | β-amyrin | -6.5 |
| 10. | α-amyrin | -6.6 |
| 11. | Uscharin | -6.7 |
| 12. | Calotropin | -7.0 |
| **Synthetic drugs** |   |   |
| 1. | Paracetamol | -3.9 |
| 2. | Ibobrufen | -4.2 |
| 3. | Indomethacin | -5.2 |
A. α-amyrin docking (whole protein) enlarge view of a. Binding 2D interaction and ligand binding position

B. Anthocyanins docking (whole protein) and enlarge view of b. Binding 2D interaction ligand binding position

C. β-amyrin docking (whole protein) and enlarge view of c. Binding 2D interaction ligand binding position

D. β-sitosterol docking (whole protein) and enlarge view of d. Binding 2D interaction ligand binding position
E. Calotropin docking (whole protein) and enlarge view of e. Binding 2D interaction ligand binding position

F. Calotropursenyl acetate docking (whole protein) and f. Binding 2D interaction enlarge view of ligand binding position

G. Lupeol docking (whole protein) and enlarge view of g. Binding 2D interaction ligand binding position

H. Methyl behenate docking (whole protein) and enlarge h. Binding 2D interaction view of ligand binding position
Figure 2. Molecular surface representation of binding between CRP and different phytochemicals (A-L) and interaction analysis (a-l) by using LigPlot

(●● = Ligand bond; ●● = Non-ligand bond; ●● = Hydrogen bond with length; ○○ = Non-ligand residues involved in hydrophobic contacts; ●●● = Corresponding atoms involved in hydrophobic contacts; ●●● = hydrophobic connections)
A. Ibobufen docking (whole protein) and enlarge view of a. Binding 2D interaction ligand binding position

B. Indomethacin docking (whole protein) and enlarge view of b. Binding 2D interaction ligand binding position

C. Paracetamol docking (whole protein) and enlarge view of c. Binding 2D interaction ligand binding position

**Figure 3.** Molecular surface representation of binding between CRP and different common anti-inflammatory drugs (A-C) and interaction analysis (a-c) by using LigPlot

(●● = Ligand bond; •• = Non-ligand bond; ••• = Hydrogen bond with length; •••• = Non-ligand residues involved in hydrophobic contacts; ••••• = Corresponding atoms involved in hydrophobic contacts; •••••• = hydrophobic connections)

4. Conclusion

In conclusion, an approach to virtual screening under computational biology along with receptor-ligand binding affinity can be an easy screening method prior to identify the efficacy of exact lead compound that has potent therapeutic efficacies without any side effects. Herein, it was observed that available phytochemicals from *C. procera* can be used in future drug designing and development as anti-inflammatory and pain relieving phytomedicine at low cost. The present work also helps to identify exact compound for future functional assay. It is suggested that the present data should be validated with *in vivo* and *in vitro* test.
Acknowledgement

Authors are thankful to all developers for present software, European Protein Data Bank for crystal structure and PubChem (Open Chemistry data base) for Canonical SMILES and CORINA software for 3D structure of all available phytochemicals and synthetic drugs used in the present work. Authors also convey their thanks to Dr. Nakul C. Maiti, Senior Scientist and Uttam Pal, Inspire Fellow, Structural Biology and Bioinformatics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, India for helping in software operation and valuable suggestions.

References

[1] Y.M. de la Torre et al., Evolution of the pentraxin family: The new entry PTX4, J. Immunol. 184 (2010) 5055-5064.
[2] M.B. Pepys, G.M. Hirschfield, C-reactive protein: a critical update, J. Clin. Invest. 111 (2003) 1805-1812.
[3] W.S. Tillet, T. Francis Jr., Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus, J. Exp. Med. 52 (1930) 561-571.
[4] M.B. Pepys, M.L. Baltz, Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein, Adv. Immunol. 34 (1983) 141-212.
[5] M.B. Pepys, The acute phase response and C-reactive protein, in: Oxford Textbook of Medicine, D.J. Weatherall, J.G.G. Ledingham D.A. Warrell (eds.) 3rd ed. Oxford, Oxford University Press, 1995, pp. 1527-1533.
[6] D. Thompson, M.B. Pepys, S.P. Wood, The physiological structure of human C-reactive protein and its complex with phosphocholine, Structure. 7(2) (1999) 169-177.
[7] S. Black, I. Kushner, D. Samols, C-reactive protein, J. Biol. Chem. 279(47) (2004) 48487-48490.
[8] A. Tomlinson et al., Cyclo-oxygenase and nitric oxide synthase isoforms in rat anti-inflammatory induced pleurisy, Br. J. Pharmacol. 113 (1994) 693-698.
[9] G. Parihar et al., Anti-inflammatory effect of Calotropis procera root bark extract, Asian Journal of Pharmacy and Life Science. 1(1) (2011) 29-44.
[10] S.H. Edwards, Chemicals mediators of inflammation, in: Anti-inflammatory agents. The Merck Veterinary Manual, 2014. Available: http://www.merckvetmanual.com/mvm/pharmacology/ anti-inflammatory_agents/chemical_mediators_of_inflammation.html#v3337363.
[11] Y. Okada et al., Genome-wide association study for C-reactive protein levels identified pleiotropic associations in the IL6 locus, Hum. Mol. Gen. 20(6) (2011) 1224-1231.
[12] C.A. Denarello, Proinflammatory cytokines, Chest. 118(2) (2000) 503-508.
[13] J-M. Zhang, J. An, Cytokines, inflammation and pain, Int. Anesthesiol. Clin. 45(2) (2007) 27-37.
[14] P. Bretscher et al., Phospholipid oxidation generates potent anti-inflammatory lipid mediators that mimic structurally related pro-resolving eicosanoids by activating Nrf2, EMBO Mol. Med. 7 (2015) 593-607.
[15] H. Tilg et al., Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells, J. Exp. Med. 178(5) (1993) 1629-1636.
[16] C. Mold et al., C-reactive protein mediates protection from lipopolysaccharide through interactions with FcγR, J. Immunol. 169(12) (2002) 7019-7025.
[17] A.J. Szalai et al., Experimental allergic encephalomyelitis is inhibited in transgenic mice expressing human C-reactive protein, J. Immunol. 168 (2002) 5792-5797.

[18] S.K. Venugopal et al., Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells, Circulation. 106(12) (2002) 1439-1441.

[19] S.P. Ballou, G. Lozanski, Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein, Cytokine. 4(5) (1992) 361-368.

[20] A.S. Reddy et al., Virtual screening in drug discovery - A computational perspective, Curr. Pro. Pept. Sci. 8(4) (2007) 329-351.

[21] A. Lavecchia, C. Di Giovanni, Virtual screening strategies in drug discovery: A critical review, Curr. Med. Chem. 20(23) (2013) 2839-2860.

[22] E. Lionta et al., Structure-based virtual screening for drug discovery: Principles, applications and recent advances, Curr. Top. Med. Chem. 14 (2014) 1923-1938.

[23] W.L. Jorgensen, The many roles of computation in drug discovery, Science. 303 (2004) 1813-1818.

[24] A.K. Sharma, R. Kharb, R. Kaur, Pharmacognostical aspects of Calotropis procera (Ait.) R. Br., Int. J. Pharm. Biol. Sci. 2(3) (2011) B480-B488.

[25] P. Chandrawat, R.A. Sharma, An overview on giant milkweed (Calotropis procera (Ait.) Ait. f.), Journal of Plant Sciences. 3(1-1) (2015) 19-23.

[26] J.S. Mossa et al., Pharmacological studies on aerial parts of Calotropis procera, Am. J. Chin. Med. 19 (1991) 223.

[27] A.C. Ranab, J.V. Kamatha, Preliminary study on antifertility activity of Calotropis procera roots in female rats, Fitoterapia. 73(1) (2002) 111-115.

[28] V.L. Kumar et al., Antioxidant and protective effect of latex of Calotropis procera against alloxan induced diabetes in rats, J. Ethnopharmacol. 102(3) (2005) 470-473.

[29] I. Zafar, L. Muhammad, J. Abdul, Anthelmintic activity of Calotropis procera (Ait.), flowers in sheep, J. Ethnopharmacol. 102(2) (2005) 256-261.

[30] M. Rajani, S.K. Gupta, Anti-tumor studies with extracts of Calotropis procera (Ait.) R.Br. root employing Hep2 cells and their possible mechanism of action, Indian J. Exp. Biol. 47(5) (2009) 343-348.

[31] O.O. Shobowale et al., Phytochemical and antimicrobial evaluation of aqueous and organic extracts of Calotropis procera ait leaf and latex, Niger. Food J. 31(1) (2013) 77-82.

[32] S. Quazi, K. Mathur, S. Arora, Calotropis procera: An overview of its phytochemistry and pharmacology, Indian Journal of Drugs. 1(2) (2013) 63-69.

[33] D.A. Brodie et al., Indomethacin-induced intestinal lesions in the rat, Toxicol. Appl. Pharmacol. 17 (1970) 615-624.

[34] I. Bjarnason et al., Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans, Gastroenterology. 104 (1993) 1832-1847.

[35] K. Higuchi et al., Present status and strategy of NSAIDs induced small bowel injury, J. Gastroenterol. 44 (2009) 879-888.

[36] K. Higuchi et al., Prevention of NSAID-induced small intestinal mucosal injury: prophylactic potential of lansoprazole, J. Clin. Biochem. Nutr. 45 (2009) 125-130.

[37] H. Matsui et al., The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine, J. Clin. Biochem. Nutr. 48(2) (2011) 107-111.
[38] N. Mascolo et al., Ethnopharmacology of *Calotropis procera* flowers, J. Ethnopharmacol. 22(2) (1998) 211-221.

[39] S. Dewan, H. Sangraula, V.L. Kumar, Preliminary studies on the analgesic activity of latex of *Calotropis procera*, J. Ethnopharmacol. 73(1-2) (2000) 307-311.

[40] C.A. Winter, E.A. Risley, C.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544-547.

[41] O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2010) 455-461.

[42] G.M. Morris et al., Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639-1662.

[43] A.C. Wallace, R.A. Laskowski, J.M. Thornton, LIGPLOT: A program to generate schematic diagrams of protein–ligand interactions, Protein Eng. 8 (1995) 127-134.

[44] A.K. Shrivastava et al., C-reactive protein, inflammation and coronary heart disease, The Egyptian Heart Journal. 67 (2015) 89-97.

[45] D. Samols, A. Agrawal, I. Kushner, Acute phase proteins, in: Cytokine Reference On-Line, M. Feldman, J.J. Oppenheim (eds.), Academic Press, London, 2002, pp. 1-16.

[46] A. Basu, A.K.N. Chaudhury, Preliminary studies on the anti-inflammatory and analgesic activities of *Calotropis procera* root extract, J. Ethnopharmacol. 31 (1991) 319-324.

[47] V.L. Kumar, N. Basu, Anti-inflammatory activity of the latex of *Calotropis procera*, J. Ethnopharmacol. 44(2) (1994) 123-125.

[48] W. Koenig et al., C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992, Circulation. 99(2) (1999) 237-242.