Full Length Research Article

SCREENING OF POSSIBLE ANTI-INFLAMMATORY LIGANDS OF *TERMINALIA CHEBULA* AGAINST THE STRESS RELATED ANAEMIA USING DOCKING STUDIES

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ARTICLE INFO	ABSTRACT
	Anaemia a chronic disorder is a result of low levels of haemoglobin. The reduction in the levels of haemoglobin leads to several other complications in the body causing acute organ failure. Stress related anaemia is also at a pace in the modern world in view of the stressed life style. Interleukins were found to be more enhanced during stress related anaemia and as such the present study was focussed mainly to lower the levels of inflammatory molecules. Possible ligands were screened from *Terminalia chebula* and docked against the inflammatory molecules. The best candidate drug was selected basing on the best fit score in patchdock. Punicalagin, an active component of the plant was found to be more active in the study and further confirmation needs to be done both at *invivo* and *invitro*.

INTRODUCTION

Anemia is a form of disease in which the normal haemoglobin level is seen to be driving down of the cut-off levels (Staubli Asobayire *et al.*, 2011). It is a widespread disease, affecting the population rapidly. Anemia leads to Chronic Kidney Disease (CKD). Other than CKD, Iron deficiency is another cause of Chronic Anemia (Mowat, 1971). The risk of postoperative morbidity and mortality increases with the high prevalence of anaemia in surgical patients (Park *et al.*, 2001). Poor nutrition and poor health leads to iron deficiency which in its severe form is the direct indicator of anemia (Mowat, 1971). Increasing cause of anemia is also the HIV/AIDS, which leads to early death (Lanaro *et al.*, 2009). Patients with active Rheumatoid arthritis are observed with anemia frequently (Staubli Asobayire *et al.,* 2001). Erythropoietin, a hormone promotes the production of red blood cells in the bone marrow. More number of red blood cells in the body have great ability to carry increased levels of oxygen to cells and tissues. Anemia of inflammation is carried out through pathogenesis by certain pro-inflammatory cytokines such as IL-2, IL-6 and TNF-alpha.

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There are reports stating that these pro-inflammatory cytokines inhibit the synthesis of erythropoietin (Anderson, 2009) leading to anemia. Hepatic hormone hepcidin plays a major role in regulating iron metabolism by binding to the iron-exporter ferroportin, it internalizes and degrades the lysosomes allowing limited iron recycling and causes synthesis of hemoglobin (Nemeth *et al.*, 2003). Hepcidin also acts as mediator for inflammation for anemia (Park *et al.*, 2001). The blockage of iron recycling in case of anemia inflammation is due to high levels of hepatic hormone hepcidin. The main cytokine triggering the hepcidin expression negatively is IL-6 causes iron deficiency eventually leading to anemic condition. Tumor necrosis factor- alpha is a cytokine produced mainly by T-cells and macrophages. The TNF-alpha has an acute pro-inflammatory activities such as activation of endothelial cells, leukocytes stimulations of macrophages, leukocyte surface molecule and endothelial receptor affinity, coagulation cascade induction, neutrophils activation and synthesis of certain proteins (Hebbel *et al.*, 2001). These factors play a major role in sickle cell anemia, as the TNF in serum was found to be in increased levels even at steady state or crisis states leading to be a characteristic events of pro inflammation (Lanaro *et al.*, 2009). Nature has presented mankind with several plants that contains a lot of herbal properties in it. These plants do contain natural substances and secondary metabolites known to be

phytochemicals that can ail diseases. Ayurveda has got the healing power around the world, mostly in ancient Indian culture and are sometimes said to be the "Mother of all healings". This herbal remedies known to be phytotherapy are used to meet the needs of primary health care in developing countries and for the discovery of modern drugs in western countries (koehn *et al.*, 2005). Therefore interest in the use of herbal medicines has been increased 80% globally in the past few decades as stated by world's health organization.

Termilania chebula a deciduous moderate tree belongs to the family combretaceae, has got its extensive use in ayurveda and homeopathic medicines. The tree is known to be Haritaki in India and found mostly in north and south India. The tree is also found in countries like Turkey, China, Myanmar, Bangladesh, Egypt, Iran etc. The leaves of the plant are elliptic rhombus with an acute tip, cordate at base; flowers are monoecious, monotonous white to yellow with strong odor. The plant consists of several biologically active substances (phytochemicals) such as chebulic acid, gallic acid, ellagic acid, tannic acid, amino acids, flavonoids like luteolin, rutins and quercetin etc (Pulliah, 1931-1934; Srivastav *et al.*, 2010). Due to the vast phytochemicals present they exhibit therapeutic properties like antioxidants (Lee HS., 2007), Antibacterial (Kannan *et al.*, 2009; Malekzadeh *et al.*, 2001), Antidiabetic (Rao *et al.*, 2006), Immunodulatory effect (Nair *et al.*, 2010). Gallic acid is a naturally occurring polyphenols or organic acids found in tea leaves, oak barks, blue berries, flax seeds, walnuts, grapes and other plants, consists of much of therapeutic properties such as anti inflammatory, anti mutagenic, antihyper cholestrolemic, radio protective (Kim *et al*, 2006; Chai *et al.*, 2010; Jagetia *et al.*, 2002; Thankur *et al.*, 1988; Vani *et al.*, 1997), hence found commonly used in pharmacological industry. The anti inflammatory activity of gallic acid revealed that this compound interferes with polymorphonuclear leukocytes and scavenging of superoxide anions takes places. The compound also interferes with the NADPH-oxidase assembly resulting in inhibition process (Kroes *et al*, 1992).

Ellagic acid is a naturally occurring polyphenolic compounds found in red fruits and berries such as strawberries, raspberries, pomegranates, craneberries, and other fruits. It is a fused four ring compound of chemical formula $C_{14}H_6C_8$. The synonyms of ellagic acid is Benzoaric acid, ellagic acid, gallogen. Ellagic acid has pharmacological activities like antioxidants, antimutagenic and anticancer properties. In cancer cells, ellagic acid prevents P53 gene destruction, binds to cancer molecule making them inactive. Chemoprotective effects against certain cancers by ellagic acid was also reported (Törrönen, 2009). Chebulic acid a polyphenolic compounds has a chemical formula of $C_{14}H_{12}O_{11}$. Chebulic acid is a isomer derived from ellagitannins such as chebulinic acid or chebulagic acid. Chebulic acid is mainly found in Termilania chebula. Ellagitannins are polyphenols belonging to the class hydrolysable tannins which can be converted to ellagic acid. They contain nutraceutical properties and biological properties like antioxidant, antimicrobial, anticancerous properties (Torronen 2009) which promote health. Chebulinic acid is a form of ellagitannin which has several biological activites including inhibition of cancer cell growth. In the present study we tried to dock the possible ligands to the inflammatory

molecules which are directly or indirectly responsible for the acute damage caused in anemia. The study used Modeller, Rampage and Patchdock softwares.

MATERIALS AND METHODS

Softwares like SWISH Model, swish PDB viewer, ACD chemSketch, Rampage, ArgusLab 4.0.1, and PATCHDOCK were used for the study. Anaemia is the result of systemic inflammation which is brought about by IL-1, IL-6, TNF and Prostaglandin E2. The same protein receptors were used in the present context, and a model study to dock or inhibit the receptors were aimed at. Human IL-1 with Accession no AAH08678.1 and human IL-6 with Accession no AD13886.1and Human Prostaglandin E2 with Accession no NP_000949.1 were taken from NCBI's entrez database. Homology modelling was done using SWISH Model freeware for depicting the structures of these receptors in 3d. The templates were designed and the best template was chosen for the docking study. For the IL-1 the following templates were used which were homologous to the given. 5i1b.1.A (identity 100%, source *Homo sapiens*), 6i1b.1.A (identity 100%, source *Homo sapiens*), 2mib.1.A (identity 100%, source *Homo sapiens*). For the IL-6 the following templates were used which were homologous to the given. 1il6.1.A (identity 100%, source *Homo sapiens*), 1alu.1 (identity 100%, source *Homo sapiens*), 4ni9.1.C (identity 100%, source *Homo sapiens*). 5 Models of the above proteins were generated using Modeller9v7. The models were further analyzed on Rampage Ramchandran plot server and the best model was selected and used for further docking studies.
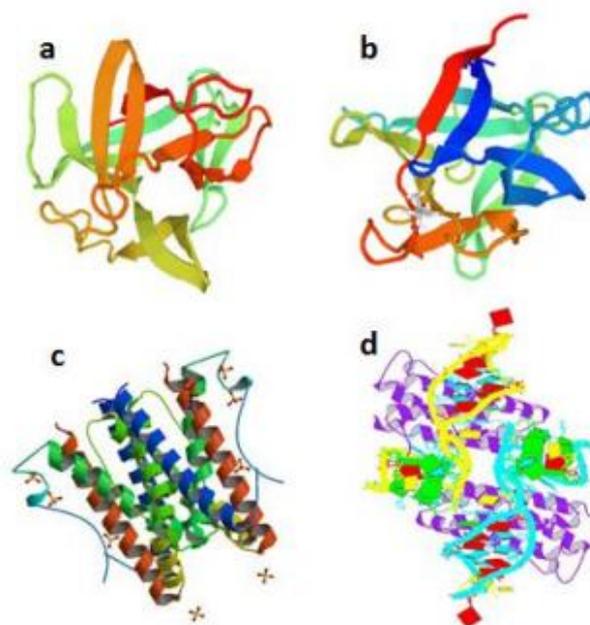

Fig 1. Picture showing the pdb structures of the best models of the receptors. A: 5i1b.1.A; B: 2mib.1.A; C: 1alu.1; D: 4ni9.1.C

The chemical structures of all the active components (Chebulagic acid, corilanin, ellagic acid, gallic acid and Punicalagin) found to be responsible for the healing of inflammation were selected drawn using ACD chemsketch software. The structures obtained were converted to *.pdb file using Argus lab software.
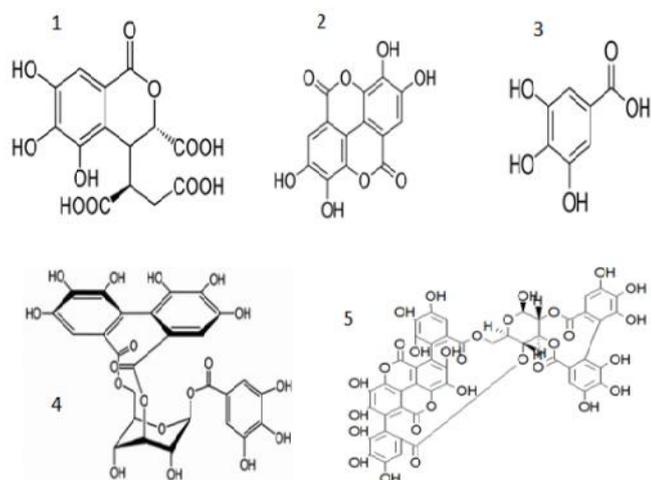
Fig. 2. Picture showing the chemical structures of the ligands of interest. 1: Chebulagic acid; 2: Ellagic acid; 3: Gallic acid; 4:Corillanin; 5: Punicalagin

Structures

All of the protein models designed for IL-1 and IL-6 of *Homo sapiens* were docked with the active components (Chebulagic acid, corilanin, ellagic acid, gallic acid and Punicalagin) of *Terminalia chebula*.

RESULTS

3D structure of the proteins for IL-1 and IL-6 obtained by homology modeling was analyzed by Rampage20. Rampage gives the best Ramachandran plot. The results obtained for best model were shown in Fig. 3.
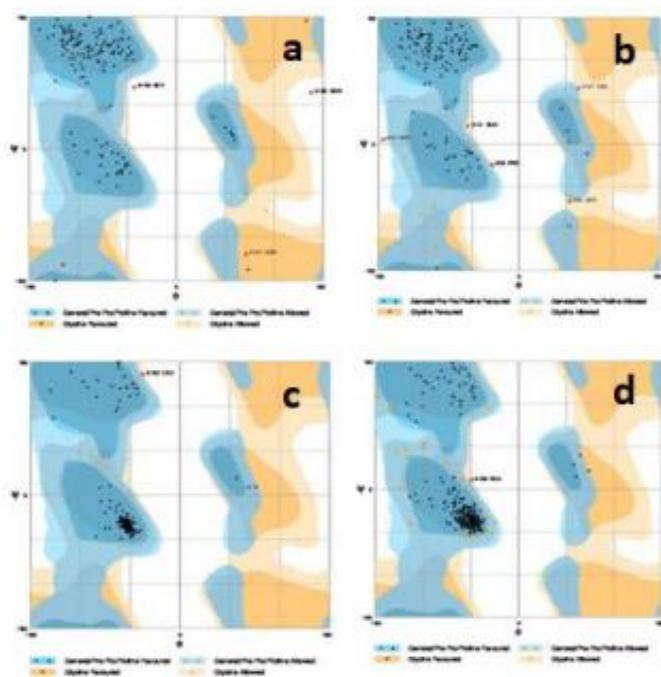

Fig. 3. Plots of Ramachandran plot showing the best fit models of the receptors. a: 5i1b.pdb; b: 2mib.pdb; c: 1alu.pdb; d: 4ni9.pdb

Table 1. Table showing the percent of residues in the favoured and allowed regions obtained from the Rampage

	5i1b.pdb	2mib.pdb	1alu.pdb	4ni9.pdb
Number of residues in favoured region (~98.0% expected)	144 (96.6%)	132 (89.8%)	149 (97.4%)	256 (91.4%)
Number of residues in allowed region (~2.0% expected)	2 (1.3%)	10 (6.8%)	3 (2.0%)	23 (8.2%)
Number of residues in outlier region	3 (2.0%)	5 (3.4%)	1 (0.7%)	1 (0.4%)

The patch Dock results seem to be promising towards the development of a new candidate drug. The successful docking of IL-1 and Il-6 with Chebulagic acid, Corilanin, Ellagic acid, Gallic acid and Punicalagin proves of the above statement. The Punicalagin seems to be more promising as per the patch dock. The scores of punicalagin for 5i1b.1.A, 2mib.1.A, 1alu.1, 4ni9.1.C are 4472, 4716, 4486 and 5040 respectively. Though the other compounds are showed good scores in relation to the docking, punicalagin seems to be better fit than the other models.
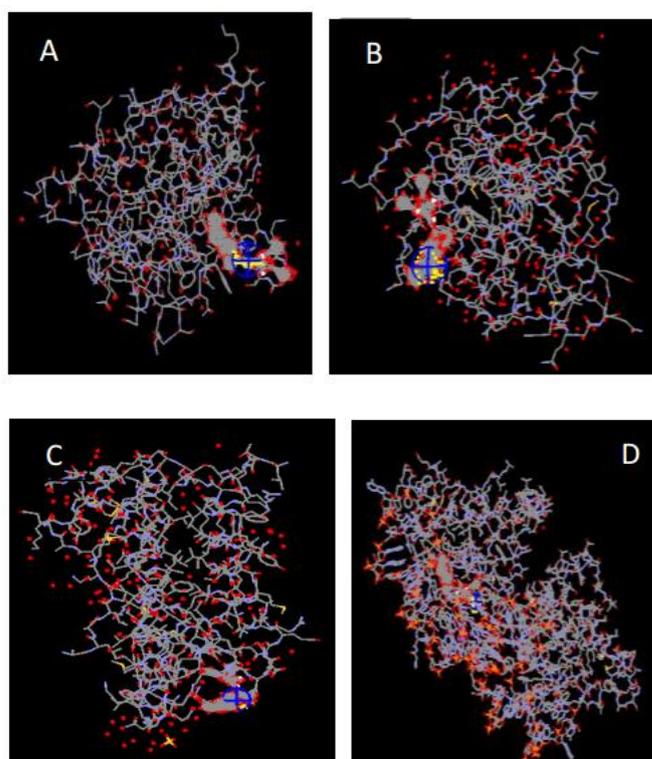

Fig 4. pictures showing the patch dock results of the proteins and ligands. Only the punicalagin pictures were shown in the paper. A: 5i1b.1.A with Punicalagin; B: 2mib.1.A with Punicalagin; C: 1alu.1 with Punicalagin; D: 4ni9.1.C with Punicalagin

Conclusion

The inflammatory molecules which are found to play an active role in inducing stress related anaemia were studied using docking. *Terminalia chebula* was used as traditional healing herb for anaemia since ages. The authors tried to use the *insilico* modelling to dock the possible ligands found in the plant against the inflammatory molecules. Among the possible ligands punicalagin was found to be more effective in terms of docking score. Though the study was completely a bioinformatics approach the results seem to be promising in identifying a potential candidate drug. However further study has to be done in isolating and purifying the said compound

and the candidate drug need to be set to clinical trials both *invivo* and *invitro*.

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