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MOLECULAR MODELING AND STRUCTURAL ANALYSIS OF ARYLESTERASE OF *ANCYLOSTOMA DUODENALE*

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ABSTRACT

Parasitic worm infection of humans is one of the most commonly prevalent helminth infection that has imposed great impact on society and public health in the developing world. The two species of hookworm, namely *Ancylostoma duodenale* and *Necator americanus* may be primarily responsible for causing parasitic infections in human beings. The highly prevalent areas for *Ancylostoma duodenale* infections are mainly India, Middle East, Australia, northern Africa and other parts of the world. The serum arylesterases/paraoxonases are family of enzymes that is involved in the hydrolysis of a number of organophosphorus insecticides to the nontoxic products. The participation of the enzymes in the breakdown of a variety of organophosphate substrates that is generally made up of paraoxon and numerous aromatic carboxylic acid esters (e.g., phenyl acetate), and hence combats the toxic effect of organophosphates. The aim of the present investigation is to evaluate the arylesterases of *Ancylostoma duodenale* giving special importance to structure generation, validation of the generated models, distribution of secondary structural elements and positive charge distribution over the structure. By the implementation of comparative modeling approach we propose the first molecular model structure of arylesterases of *Ancylostoma duodenale*.

KEYWORDS: ***Ancylostoma duodenale,*** **Arylesterases, Molecular model, Paraoxonase, Helminthiasis, Protein structure.**

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INTRODUCTION

Human helminthiasis is a common helminth infection that has predominant implications on socioeconomic and public health. It is infecting an estimated 600 million persons worldwide, resulting in approximately 135,000 deaths annually [1, 2]. The causative agent of human infection is primarily two species of hookworm, namely *Ancylostoma duodenale* and *Necator americanus* [3]. *A. duodenale* infections are distributed geographically mainly in the India, Middle East, Australia, northern Africa, Australia, and Europe, while the distribution of *N. americanus* is mostly found in the Western Hemisphere, eastern Asia, sub-Saharan Africa, and southeast Asia [4]. Furthermore, zoonotic helminths such as *Ancylostoma ceylanicum*, *Ancylostoma braziliense*, and *Ancylostoma caninum* have been mentioned as potentially important public health perils in numerous areas [5, 6]. Adult (Ad) hookworms live in the small intestine of the host and lays eggs which pass in the feces and eventually hatch in the soil. The first stage larva (L1) feeds on soil bacteria and ultimately molts twice to produce the non-feeding, infective third stage larva (iL3). iL3 penetrate the host via skin, or through oral route in the case of *Ancylostoma* species, that molts twice, and matures to Ad in the small intestine. *A. duodenale* and *A. caninum* L3s may also cause an infection in the host, aborts maturation temporarily and enters an arrested state (hypobiosis) within the somatic tissues of the host [7], which gets reactivated upon receiving signal by the physiological changes in the host such as pregnancy [8].The serum arylesterases/paraoxonases are a group of enzymes that catalyses the hydrolysis of the toxic metabolites produced by a variety of organophosphorus insecticides. The enzymes hydrolyse a large number of organophosphate substrates that comprises of paraoxon and a number of aromatic carboxylic acid esters (e.g., phenyl acetate), and therefore provides resistance to organophosphate toxicity [9]. Mammals possess 3 distinct Arylesterase/Paraoxonase types, termed Paraoxonase 1-3 (PON1-3) [9, 10]. In case of mice and humans, the PON genes are present in close proximity on the same chromosome. Arylesterase protein is also found in *Ancylostoma duodenale* parasite. The Arylesterase/Paraoxonase proteins are highly potential molecular and physiological targets for developing chemical control of *A. duodenale* as well as for the development of vaccines.The consequences of the second most common parasitic infections caused by nematodes on PON1 activity were investigated by Farid et al. [11, 12], who put forth that infection by *Nippostrongylus brasiliensis*, a gastrointestinal nematode that causes infection in mice and rats and has a life cycle similar to human pathogens *Ancylostoma duodenale* and *Necator americanus*, diminishes the activity of serum PON1 in male rats [13]. Investigations by the same group revealed that *N. brasiliensis* infection in rats fed a high-fat diet led to a decline in serum PON1 activity in association with an atherogenic lipid profile [14]. In another study reported by Helmy et al. [15], it was shown that serum and liver arylesterase and paraoxonase activities were decreased significantly in mice at 10 weeks after parasitic infection with *S. mansoni*, in comparison to uninfected healthy mice. Due to this background the intent of this study was to determination of a homology based structure of arylesterase of *Ancylostoma duodenale*. The additional objective was to examine the charge distribution over the structure and distribution of secondary structural elements with the aid of *In silico* based approach. The generated and validated structure thus may be used in future as a template for vaccine development and insecticide development.

MATERIALS AND METHODS

Amino acid sequence of arylesterase of *Ancylostoma duodenale* was obtained from National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov) [16]. Comparative molecular model of arylesterase of *Ancylostoma duodenale* was created with the help of Swiss-PDB Viewer and iterative implementation of the threading assembly refinement algorithm [17, 18]. Energy minimization step for structural improvements of molecular model of arylesterase of *Ancylostoma duodenale* was performed by Swiss-PDB Viewer and ModRefiner tool [19, 20]. For the validation of structural model obtained by comparative modeling strategy was analyzed by PROCHECK algorithm, ProSA and ProQ tool [21, 22, 23]. Location of structural components and distribution of positive charge over the structure was performed with the aid of UCSF Chimera package [24, 25].

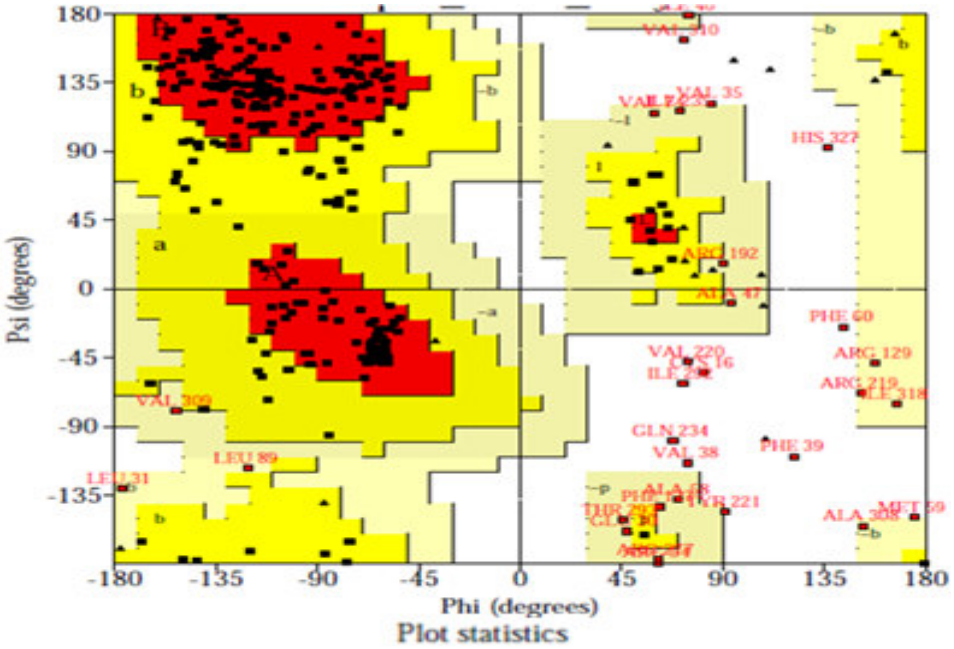

Figure 1

Ramachandran plot analysis of molecular model of arylesterase of Ancylostoma duodenale.

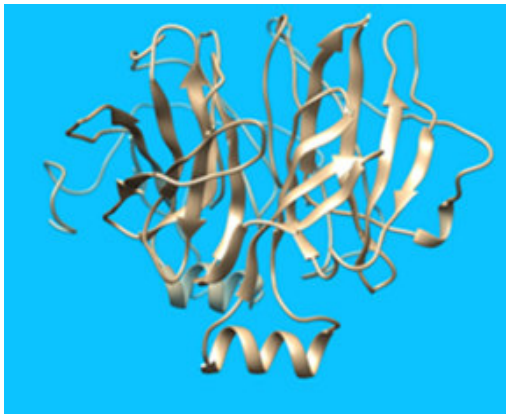

Figure 2

Three-dimensional modeled structure of arylesterase of Ancylostoma duodenale.

Figure 3
Three-dimensional modeled structure of arylesterase of Ancylostoma duodenale (top view).

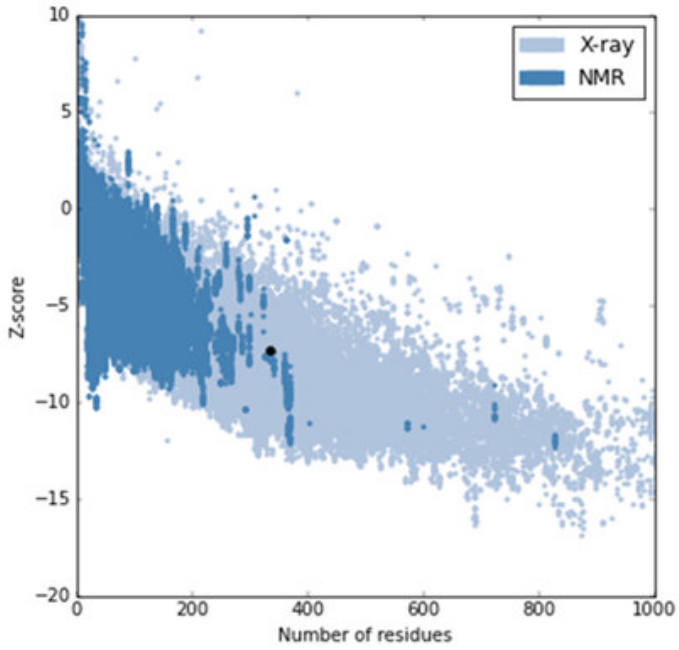

Figure 4
Stereo-chemical validation of modeled structure of arylesterase of Ancylostoma duodenale.

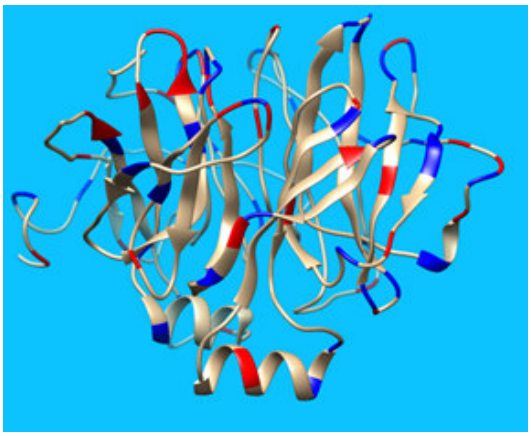

Figure 5
Positive charge distributions over the modeled structure of arylesterase of Ancylostoma duodenale.

RESULTS AND DISCUSSION

Clinically, infection in human may lead to iron-deficiency anemia, resulting in mental retardation and growth deficiencies, especially in children [26]. Diagnostic analysis by identifying and differentiating the species involved is essential in carefully monitoring the efficacy of mass treatment and effective control of hookworm infection [2]. The treatment of a population with anthelminthic drugs is performed frequently without

identifying the causative species of infection. It has been suggested through the reports that a clinical manifestation such as severity of anemia varies according to the hookworm species involved in the infection while the route of infection for each hookworm species spreads the infection via different routes (e.g., *N. americanus* infection occurs mainly via skin penetration , whereas *Ancylostoma spp.* infections spread commonly by ingestion of infective third-stage larvae), identifation of the species is the most important step in designing strategies for appropriate and effective prevention and control [27]. In addition, if the prevalent infectious agent is a zoonotic hookworm, the control target and strategies designed require encompass through animal hosts. Host immune system plays a vital role in fighting against parasitic infections, but it also has some negative impact on human body [28]. For instance, generation of oxidative stress is a crucial factor which is responsible for immune activation [29]. The generation of oxidants at the time of parasitic infection occurs via three major pathways: initially, they are released by immune cells which, utilizes their cytotoxic effects to combat the pathogen; second, oxidants are generally by-products of oxygen consumption, and enhanced metabolism during an immune response may result into the generation of additional toxic oxidants; and third, parasites may also release oxidants directly via degradation products of their own metabolic activity. Non-targeting toxic oxidants aids in protecting immune system and possesses a negative side-effect by destroying normal host tissues and blocking their healthy functioning [30]. Ramachandran plot evaluation (PROCHECK analysis) is a benchmark for validation purpose of protein structure models [31]. Ramachandran plot for arylesterase of *Ancylostoma duodenale* has been illustrated in Figure 1. Altogether 96% of the residues were detected in allowed and favored regions, which in turn validate the quality of the generated protein structural model. PROCHECK algorithm also displayed 70.8% of residues in the most favored regions, with 19.3% residues in additionally allowed regions, respectively (Fig 1). This demonstrated that the backbone dihedral angles, phi and psi, in the arylesterase, were reasonably accurate. This proposes that the modeled structure of arylesterase of *Ancylostoma duodenale* is satisfactory and acceptable (Fig 2 and Fig 3). As shown in Figure 4 the Z-score (ProSA tool) of arylesterase of *Ancylostoma duodenale* was – 7.32. The score was well inside the range of scores usually observed for proteins of matching size indicating highly reliable structures. The ProQ algorithm also indicates a good quality of the molecular model of arylesterase of *Ancylostoma duodenale* with an LGscore of 4.389 and MaxSub score of 0.349. The manual inspection of arylesterase of *Ancylostoma duodenale* postulates that the total protein is composed by 336 numbers of amino acids. The presence of total number of positively charged amino acids is 33. In comparison to that the total number of negatively charged amino acids is 37 (Fig 5).

CONCLUSION

In the current study, we have successfully utilized comparative modeling approach to propose the first molecular model structure of arylesterase of *Ancylostoma duodenale*. The present research work emphasizes on gathering knowledge of evolutionary structural enrichment strategy of arylesterase of *Ancylostoma duodenale*. The existence of various secondary structural element over structure renders the molecule specific peculiarity of arylesterase of *Ancylostoma duodenale*.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

REFERENCES

1. Hotez PJ. One world health: neglected tropical diseases in a flat world. PLoS Negl Trop Dis. 2009; 3:e405.
2. Ngui R, Ching LS, Kai TT et al. Molecular identification of human hookworm infections in economically disadvantaged communities in Peninsular Malaysia. Am J Trop Med Hyg. 2012; 86:837-842.
3. Chan MS, Medley GF, Jamison D et al. The evaluation of potential global morbidity attributable to intestinal nematode infections. Parasitology. 1994; 109 (Pt 3):373-387.
4. de Silva NR, Brooker S, Hotez PJ et al. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol. 2003; 19:547-551.
5. Mahdy MA, Lim YA, Ngui R et al. Prevalence and zoonotic potential of canine hookworms in Malaysia. Parasit Vectors. 2012; 5:88.
6. Phosuk I, Intapan PM, Thanchomnang T et al. Molecular detection of *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, and *Necator americanus* in humans in northeastern and southern Thailand. Korean J Parasitol. 2013; 51:747-749.
7. Mitreva M, McCarter JP, Arasu P et al. Investigating hookworm genomes by comparative analysis of two *Ancylostoma* species. BMC Genomics. 2005; 6:58.
8. Stone WM, Girardeau M. Transmammary passage of *Ancylostoma caninum* larvae in dogs. J Parasitol. 1968; 54:426-429.

9. Primo-Parmo SL, Sorenson RC, Teiber J et al. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics. 1996; 33:498-507.
10. Billecke S, Draganov D, Counsell R et al. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. Drug Metab Dispos. 2000; 28:1335-1342.
11. Farid AS, Nakahara K, Murakami N et al. Decreased serum paraoxonase-1 activity during intestinal nematode (*Nippostrongylus brasiliensis*) infection in rats. Am J Trop Med Hyg. 2008; 78:770-776.
12. Farid AS, Horii Y. Modulation of paraoxonases during infectious diseases and its potential impact on atherosclerosis. Lipids Health Dis. 2012; 11:92.
13. Harcus YM, Parkinson J, Fernandez C et al. Signal sequence analysis of expressed sequence tags from the nematode *Nippostrongylus brasiliensis* and the evolution of secreted proteins in parasites. Genome Biol. 2004; 5:R39.
14. Farid AS, Mido S, Linh BK et al. An atherogenic lipid profile with low serum paraoxonase-1 activity during nematode infection in rats. Eur J Clin Invest. 2010; 40:984-993.
15. Helmy MM, Mahmoud SS, Fahmy ZH. *Schistosoma mansoni*: effect of dietary zinc supplement on egg granuloma in Swiss mice treated with praziqantel. Exp Parasitol. 2009; 122:310-317.
16. Sayers EW, Barrett T, Benson DA et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2012; 40:D13-25.
17. Guex N, Peitsch M. Swiss-PdbViewer: a fast and easy-to-use PDB viewer for Macintosh and PC. Protein Data Bank Quaterly Newsletter. 1996; 77.
18. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics. 2008; 9:40.
19. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis. 1997; 18:2714-2723.
20. Xu D, Zhang Y. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. Biophys. J 2011; 101:2525-2534.
21. Laskowski RA, Rullmannn JA, MacArthur MW et al. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J Biomol NMR. 1996; 8:477-486.
22. Sippl MJ. Recognition of errors in three-dimensional structures of proteins. Proteins. 1993; 17:355-362.
23. Wallner B, Elofsson A. Can correct protein models be identified? Protein Sci. 2003; 12:1073-1086.
24. Pettersen EF, Goddard TD, Huang CC et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004; 25:1605-1612.
25. Goddard TD, Huang CC, Ferrin TE. Visualizing density maps with UCSF Chimera. J Struct Biol. 2007; 157:281-287.
26. Albonico M, Crompton DW, Savioli L. Control strategies for human intestinal nematode infections. Adv Parasitol. 1999; 42:277-341.
27. Beaver PC, Jung RC, Cupp EW et al. (1984) Clinical parasitology. Lea & Febiger.
28. Kamble P, Bhagwat V, Trivedi DJ, Bargale A. Comparative study of free radical activity in *Plasmodium falciparum* and *Plasmodium vivax* malaria patients. Int J Pharm Bio Sc. 2011; 2:99-102.
29. Dowling DK, Simmons LW. Reactive oxygen species as universal constraints in life-history evolution. Proc Biol Sci. 2009; 276:1737-1745.
30. van de Crommenacker J, Richardson DS, Koltz AM et al. Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. Proc Biol Sci. 2012; 279:1466-1476.
31. Panda S, Kumari L, Panda S. Structural understanding of Cytotoxin 1 of *Naja sputatrix*: a potential anticancer agent. J Drug Deliv Ther. 2016; 6:59-63.