International Journal of Applied
and Natural Sciences (IJANS)
ISSN (P): 2319–4014; ISSN (E): 2319–4022
Vol. 9, Issue 3, Apr–May 2020; 59–68

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

COMPUTATIONAL SCREENING OF ANTI-TOXIC COMPOUNDS FROM INDIAN MEDICINAL PLANTS AGAINST ENTEROTOXEMIA IN SHEEP AND GOAT: AN IMMUNE-INFORMATICS APPROACH

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ABSTRACT

Enterotoxemia is also called as the pulpy kidney disease or over eating disease which is an acute poisonous condition. It is caused by the non motile anaerobic spore forming bacterium called Clostridium perfringens. As the organism grows in number, it releases very potent toxins (bacterial poisons) that harm the animal. These toxins can cause damage to the intestine as well as numerous other organs. This can result in fatalities, particularly in the non-vaccinated animal or in the newborn lamb or kid whose dam has not been vaccinated. An attempt was undertaken to find the potential drug candidates using Indian medicinal plants against enterotoxemia using the immune-bioinformatics approach. The study showed that the plant Andrographis paniculata (Nilavembu) showed very good interaction with the target protein. However the results could be validated with in-vivo and in-vitro studies.

***KEYWORDS**: Enterotoxemia, Andrographis, In-Silico*

Article History

***Received:** 16 Apr 2020 | **Revised:** 18 Apr 2020 | **Accepted:** 04 May 2020*

INTRODUCTION

Enterotoxemia is a frequently severe disease of sheep and goats of all ages. It is caused by two strains of bacteria called *Clostridium perfringens* – the strains are termed types **C and D.** These bacteria are normally found in low numbers in the gastrointestinal tract of all sheep and goats.

These organisms are normally “laying low” in the small and large intestine – that is, they are present in relatively low numbers and appear to be in a relatively quiescent state in the normal, healthy animal. What appears to trigger them to cause disease is a *change in the diet* of the animal. Most commonly, the change that triggers disease is an increase in the amount of grain, protein supplement, milk or milk replacer (for lambs and kids), and/or grass that the sheep or goat is ingesting. Collectively, these feeds are rich in starch, sugar, and/or protein. When unusually high levels of these nutrients reach the intestine, *Clostridium perfringens* undergoes explosive growth, increasing its numbers rapidly within the intestine. As the organism grows in number, it releases very potent toxins (bacterial poisons) that harm the animal. These toxins can cause damage to the intestine as well as numerous other organs. This can result in fatalities, particularly in the non-vaccinated animal or in the newborn lamb or kid whose dam has not been vaccinated.

Enterotoxemia is also called as the pulpy kidney disease or over eating disease which is an acute poisonous condition. It is caused by the non motile anaerobic spore forming bacterium called *Clostridium perfringens.*

Clostridium perfringens is a Gram – positive sporulated, anaerobic bacterium that is one of the most important causes of clostridial enteric disease in domestic animals (Kalender, H *et al.*, 2006). *Clostridium perfringens* consists of 5 types A, B, C, D and E. The organism can produce up to 16 different toxins and enzymes in various combinations, including lethal toxins such as alpha (α), beta (β), epsilon (ε) and iota (ι). Toxin typing of *C. Perfringens* is important since particular toxin types are associated with specific enteric diseases in animals (Uzal, F.A *et al.*, 2014). Among the 5 types of *Clostridium perfringens* type A, C and D causes enterotoxemia in cattle. Prevalence of *C. perfringens* type C was reported for the first time in India. *Clostridium perfringens* type D where found to be the major causative types for enterotoxemia in sheep and goat (Kumar, N.V *et al*., 2014). C. Perfringens type A is commonly recovered from the intestinal tracts of animals, while other types, B, C, D, and E, are less common in the intestinal tracts of animals (Songer, J.G., 1996).

Etiology and Pathogenesis

The causative agent is *C perfringens* type D. Predisposing factors are essential, the most common being the ingestion of excessive amounts of feed or milk in the very young and of grain in feedlot lambs. In young lambs, the disease usually is restricted to ewes with single lambs, because ewes with twins seldom give enough milk to allow enterotoxemia to develop. In the feedlot, the disease usually is seen in lambs that switched rapidly to high-grain diets. As starch intake increases, it provides a suitable medium for overgrowth of *C perfringens*, producing epsilon toxin. The toxin causes vascular damage, particularly in capillaries of the brain. Many adult sheep carry strains of *C perfringens* type D as part of their normal intestinal microflora, which is the source of organisms that infect the newborn. Most such carriers have nonvaccinal antitoxin serum titers.

Enterotoxemia affects all ages of the cattle and also affects sheep, goats and ruminants. They always affect better conditioned animals. The bacterium multiplies and produces the toxin inside the body, resulting in killing of the infected animal. The *Clostridium perfringens* is the normal inhabitants or occupants of the intestine in low in number. Usually these organisms produce little toxins, under normal conditions the toxins produced by the organisms gets neutralized or removed with the help of the gut movements or inactivated by certain circulating antibodies.

Abrupt changes in animal's diet such as increase in the protein supplement, carbohydrates, milk or milk replacer and grazing of grass that is ingested by the animal etc., these feeds are rich in starch, sugar and protein. When the nutrient content reaches the intestine, the organisms undergo an explosive growth, and then it is multiplied and increased in number in the intestine. The organism releases the very potent bacterial poisonous toxins which can damage the intestine as well as other organs. Since amount of toxin released is increased so the amount of neutralizing rate is comparatively slower than that of toxin produced (NSW department Primefact 418, 2007)

Clinical Findings

Usually, sudden deaths in the best-conditioned lambs are the first indication of enterotoxaemia. In some cases, excitement, in coordination, and convulsions occur before death. Opisthotonos, circling, and pushing the head against fixed objects are common neurologic signs; frequently, hyperglycemia or glycosuria is present. Diarrhea may or may not develop. Occasionally, adult sheep are affected too, showing weakness, in coordination, convulsions, and death within 24 hr. In goats, the course of disease ranges from peracute to chronic, with signs that vary from watery diarrhea with or without

blood to sudden death. Affected calves not found dead show mania, convulsions, blindness, and death within a few hours. Subacutely affected calves are stuporous for a few days and may recover. In goats, diarrhea and nervous signs are seen, and death may occur over several weeks. Type D enterotoxemia occasionally is seen in young horses that have overeaten.

Lesions

Necropsy may reveal only a few hyperemic areas on the intestine and a fluid-filled pericardial sac. This is particularly the case in young lambs. In older animals, hemorrhagic areas on the myocardium may be found as well as petechiae and ecchymoses of the abdominal muscles and serosa of the intestine. Bilateral pulmonary edema and congestion frequently occur but usually not in young lambs. The rumen and abomasum contain an abundance of feed, and undigested feed often is found in the ileum. Edema and malacia can be detected microscopically in the basal ganglia and cerebellum of lambs. Rapid postmortem autolysis of the kidneys has led to the popular name of pulpy kidney disease; however, pulpy kidneys are by no means always found in affected young lambs and are seldom found in affected goats or cattle. Hemorrhagic or necrotic enterocolitis may be seen in goats.

Diagnosis

A presumptive diagnosis of enterotoxemia is based on sudden, convulsive deaths in lambs on carbohydrate-rich feed. Smears of intestinal contents reveal many short, thick, gram-positive rods. Confirmation requires demonstration of epsilon toxin in the small-intestinal fluid. Fluid, not ingesta, should be collected in a sterile vial within a few hours after death and sent under refrigeration to a laboratory for toxin identification. Chloroform, added at 1 drop for each 10 mL of intestinal fluid will stabilize any toxin present. Although immunologic tests have been developed to replace the traditional mouse assay for detection of toxin, they are less sensitive. A PCR for detection of epsilon toxin gene is available for identification of the isolates as either type B or D.

Signs and Symptoms

Mostly animals simply found dead once they are affected by the *Clostridium perfringens*. Some of the acute cases show signs of stomach pain, diarrhoea, blood traces in the stool and nervous problems. They lose their ability to stand and become weakened.

The signs of enterotoxemia in sheep and goats include:

- The animals may abruptly go off of feed and become lethargic.
- Affected animals may show signs of stomach pain, such as kicking at their belly, repeatedly laying down and getting up, laying on their sides, panting, and crying out.
- Diarrhea may develop; in some cases, there is blood visible in the loose stool.
- Animals may lose the ability to stand, lay on their sides, and extend their legs, with their head and neck extended back over their withers. This posture is caused by the effects of the toxins on the brain. Death commonly occurs within minutes to hours after this sign is seen. Because enterotoxemia can progress so quickly, animals may be found dead with no previous signs of disease.

Treatment

Since the treatment in severe cases is unsuccessful, there is no treatment for curing enterotoxemia. Only the supportive treatment is available such as probiotics, analgesics, oral electrolyte solution, intravenous fluids, antibiotic therapy, supplement oxygen and antisera (D. Van Metre, 2010). Immuno-bioinformatics An attempt was undertaken to fund novel drug candidates for the treatment of enterotoxemia.

MATERIALS AND METHODS

In – Silico Analysis

In – silico approach in drug formulations against Enterotoxemia in cattle have potential to increase the rate of discovering new drug while decreasing the requirement for costly lab work and clinical preliminaries. The drugs are produced and screened in effective way. In this study, the target protein is modelled and drug like compound structures were downloaded from the chemical databases and were docked in commercial software – Accelerys Discovery Studio.

TARGET PROTEIN

The Target protein was identified as the Epsilon Toxin (ETX) based on the documentary sources.

Epsilon Toxin (ETX)

The Enterotoxemia is one of the Gastro-intestinal diseases caused by the Clostridium perfringens. The type D Clostridium perfringens produces Epsilon Toxin (ETX). But there is no much information about the effects of epsilon toxin. This toxin may cause neurological signs and pulmonary edema. In the brain, the ETX affects the endothelial cells and leads to perivascular edema and its consequent degeneration causes necrosis in ceberal parenchyma. They act directly on the neurons. So based on the above evidence, epsilon toxin is the major responsible for the causing infection in ruminants.

UNIPROT

UNIPROT - the primary sequence database which contains an outsized quantity of knowledge regarding the biological operations of proteins derived from the analysis of literature. From the literary sources, the target protein Epsilon toxin (ETX) is identified. The sequence of the epsilon toxin (ETX) of *Clostridium perfringens* Type D is retrieved from the UNIPROT database. The protein sequence of the epsilon toxin (ETX) of *Clostridium perfringens* Type D is given here under:

```
>tr|B1V315|B1V315_CLOPF Epsilon-toxin OS=Clostridium perfringens D str.
JGS1721 OX=488537 GN=CJD_A0448 PE=4 SV=1
MKKNLVKSLAIASAVISIYSIVNIVSPTNVIAKEISNTVSNEMSKKASYDNVDTLIEKGR
YNTKYNYLKRMEKYYPNAMAYFDKVTINPQGNDFYINNPKVELDGEPSMNYLEDVYVGKA
LLTNDTQQEQKLKSQSFTCKNTDTVTATTTHTVGTSIQATAKFTVPFNETGVSLTTSYSF
ANTNTNTNSKEITHNVPSQDILVPANTTVEVIAYLKKVNVKGNVKLVGQVSGSEWGEIPS
YLAFPRDGYKFSLSDTVNKSDLNEDGTININGKGNYSAVMGDELIVKVRNLNTNNVQEYV
IPVDKKEKSNDSNIVKYRSLSIKAPGIK
```

Figure 1: Protein Sequence of Epsilon Toxin (ETX) of Clostridium Perfringens Type D.

I – TASSER

I – TASSER - Iterative Threading Assembly Refinement is the protein structure and function prediction online server under Yang Zhang's research group University of Michigan. They identify templates from PDB and models were

constructed by iterative template-based fragment assembly simulations. The sequence of Epsilon Toxin (ETX) is modelled using I – Tasser. Out of 5 models, best reliable model is selected for the interaction.

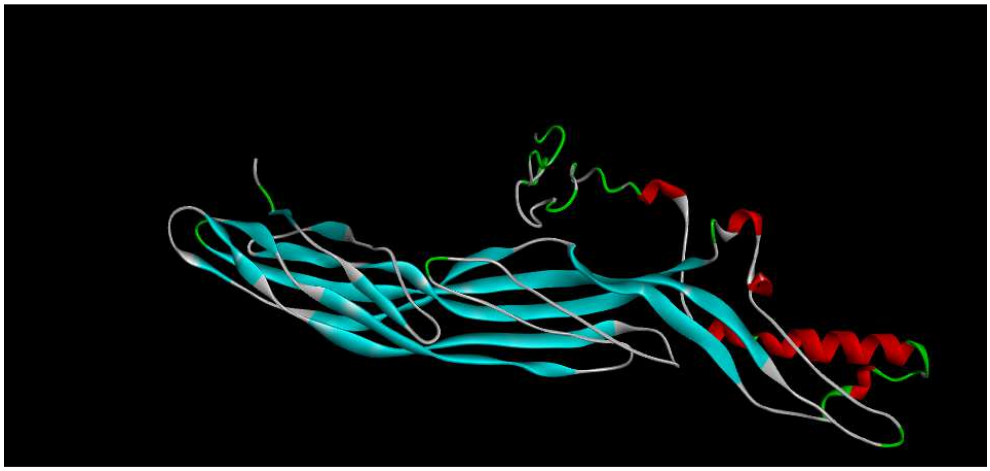

Figure 2:

LIGAND:

The Indian medicinal plants which holding the capacity of restricting the effect of the epsilon toxin released by the clostridium perfringens causing Enterotoxemia were selected from the available literature.

IMPPAT:

Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT), A curated database which has been constructed via literature, manual curation of information gathered from traditional Indian medicine, and other existing database resources. Here the .SDF, .PDB structures and Smiles, were collected.

Andrographis Paniculata (Nilavembu)

Andrographis paniculata (Nilavembu) is one such plant belonging to the family Acanthaceae known to be one of the important herbal medicines used for centuries in Asia to treat several diseases such as gastro-intestinal tract and upper respiratory infections, fever and herpes. It is known to a predominant constituent in 26 Ayurvedic formulations (Sattayasai et al., 2010).

Phytochemicals

The **Andrographis paniculata** has phytochemicals of about 31 which has been described here under.

Table 1:

Sl.No.	Name of the Phytochemical	3 D structure	SMILES format	MOL format

Among the phytochemicals Andropanoside showed more interaction and docked in 3 poses

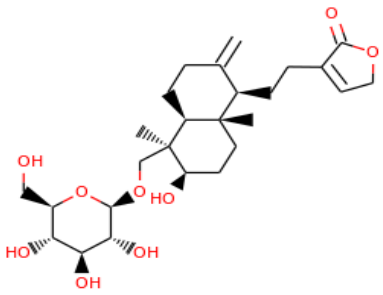

Figure 3: Structure of Andropanoside.

MOLECULAR DOCKING

Molecular docking has become an increasingly important tool for drug discovery (Xuan-Yu Meng *et al*., 2011). The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes (BJ McConkey *et al.*, 2002). Here the target protein Epsilon toxin and drug candidate Andrographis paniculata is docked using the commercial software.

Accelyrs Discovery Studio

The ligand and Receptor is docked using the Discovery studio software. Accelrys (NASDAQ: ACCL) is a software company headquartered in the US with representation in Europe and Japan. It provides software for chemical research especially in the areas of drug discovery and materials science. The ligand is docked with the receptor and the results were obtained and analysed with the help of the Discovery studio visualizer.

RESULTS

Molecular docking between drug candidate and target gives results as, the phytochemical Neo-andrographolide shows more interaction towards the epsilon toxin (ETX).

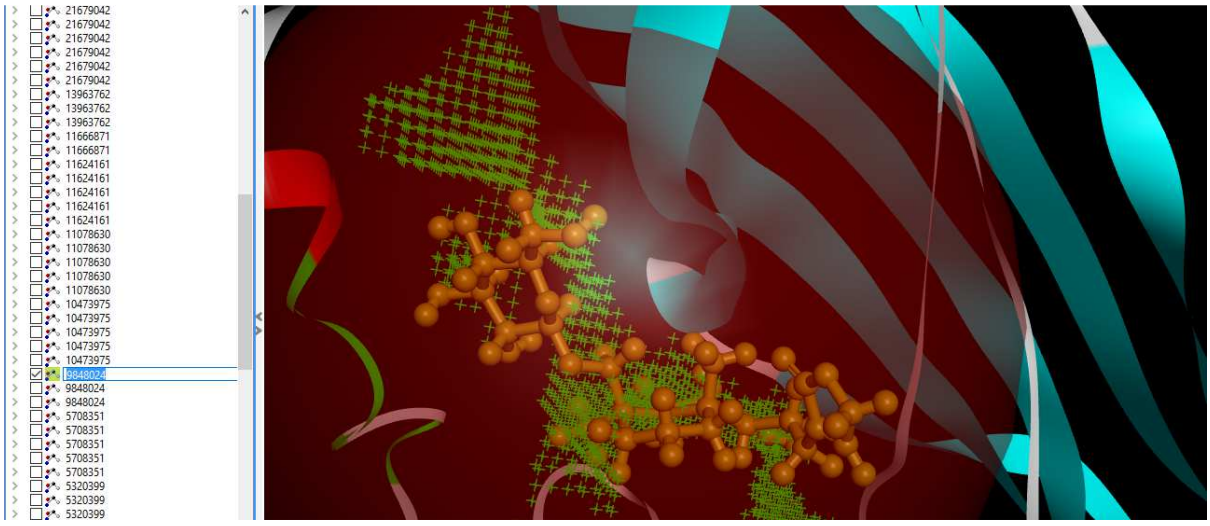

Figure 4: 3D Interaction.

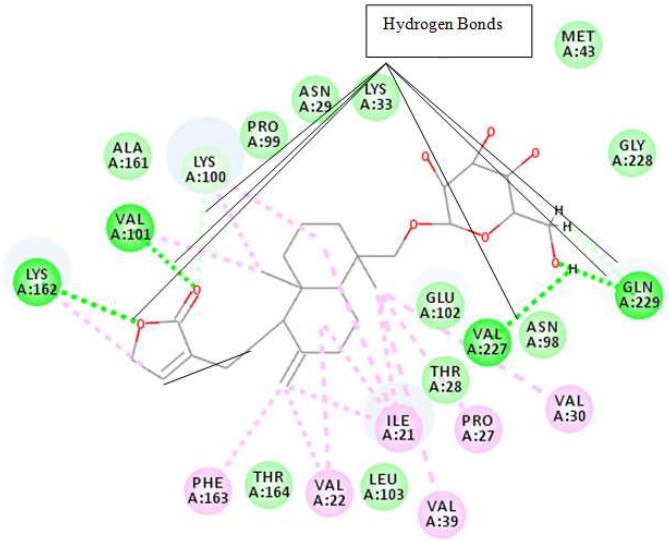

Figure 5: 2D Interaction.

PHARMACOKINETICS AND PHARMACO DYNAMICS RESULT

The Smiles of the compound Neo-andrographolide is downloaded. Then, ADME properties and Toxicity is checked with the help of SwissADME and Toxtree tools, then the results were noted.

Swiss ADME

Table 2:		
Physicochemical Properties		
1	Hydrogen Bond Acceptor	6
2	Hydrogen Bond Donor	1
3	Molar Refractivity	92.19
4	TPSA	66.38 Å
Lipophilicity		
1	Consensus Log $P_{o/w}$	2.90
2	Log $P_{o/w}$ (iLOGP)	2.77
3	Log $P_{o/w}$ (XLOGP3)	3.38
5	Log $P_{o/w}$ (MLOGP)	2.38
6	Log $P_{o/w}$ (SILICOS-IT)	3.46
7	Log $P_{o/w}$ (WLOGP)	2.51
Water Solubility		
1	Log *S* (ESOL)	-4.26
	Solubility	1.97e-02 mg/ml ; 5.54e-05 mol/l
	Class	Moderately soluble
2	Log *S* (Ali)	-4.45
	Solubility	1.26e-02 mg/ml ; 3.53e-05 mol/l
	Class	Moderately Soluble
3	Log *S* (SILICOS-IT)	-4.77
	Solubility	6.07e-03 mg/ml ; 1.70e-05 mol/l
	Class	Moderately soluble
Pharmacokinetics		
1	GI Absorption	High
2	BBB permeant	Yes
3	P-gp substrate	No
4	CYP1A2 inhibitor	Yes
5	CYP2C19 inhibitor	Yes
6	CYP2C 9 inhibitor	Yes

7	CYP2D6 inhibitor	Yes
8	CYP3A4 inhibitor	Yes
9	Log K_p (skin permeation)	-6.07 cm/s
Drug likeness and Medicinal Chemistry		
1	Lipinski Rule	0 Violations
2	PAINS	0 Alert
3	Brenk alert	0 alert
4	Lead likeness	No
5	Synthetic accessibility	3.90
6	Bioavailability Score	0.55
7	Boiled EGG	Molecule in yellow part of the egg.

TOXICITY PREDICTIONS

Toxicity was predicted with the help of the tool **TOXTREE**. The toxtree results showed that, Neo - andrographolide does not contain functional groups associated with enhanced toxicity.

SUMMARY

The Enterotoxemia or Pulpy kidney is caused by *Clostridium perfringens*. Treatment was unsuccessful in severe cases and leads to death. In this study, the antibacterial Indian medicinal plants were collected and screened the ligand and Receptor (target protein) interactions. The result states that phytochemical neo-andrographolide from the plant *Andrographis paniculata* shows more inhibition towards the target protein Etx Epsilon toxin. So, the plant *Andrographis paniculata* might be used as a drug to neutralize the harmful toxin released inside the body of the affected animal.

ACKNOWLEDGEMENT

The authors wish to thank the Tamil Nadu Veterinary and Animal Sciences University for constant support and providing insilico lab to carry out this study.

REFERENCES

1. *Bokori-Brown, M., Hall, C.A., Vance, C., da Costa, S.P.F., Savva, C.G., Naylor, C.E., Cole, A.R., Basak, A.K., Moss, D.S. and Titball, R.W., 2014. Clostridium perfringens epsilon toxin mutant Y30A-Y196A as a recombinant vaccine candidate against enterotoxemia. Vaccine, 32(23), pp.2682-2687.*
2. *Garcia, J.P., Adams, V., Beingesser, J., Hughes, M.L., Poon, R., Lyras, D., Hill, A., McClane, B.A., Rood, J.I. and Uzal, F.A., 2013. Epsilon toxin is essential for the virulence of Clostridium perfringens type D infection in sheep, goats, and mice. Infection and immunity, 81(7), pp.2405-2414.*
3. *Jemal, D., Shifa, M. and Kebede, B., 2016. Review on pulpy kidney disease. J. Vet. Sci. Technol, 7(5), p.361.*
4. *Kalender, H., Kiliç, A. and Atil, E., 2007. Enterotoxemia in a cow due to Clostridium perfringens type A Turkish Journal of Veterinary and Animal Sciences, 31(1), pp.83-84.*
5. *Kumar, N.V., Sreenivasulu, D. and Reddy, Y.N., 2014. Prevalence of Clostridium perfringens toxin genotypes in enterotoxemia suspected sheep flocks of Andhra Pradesh. Vet World, 7(12), pp.1132-1136.*
6. *McConkey, B.J., Sobolev, V. and Edelman, M., 2002. The performance of current methods in ligand–protein docking. Current Science, pp.845-856.*

7. *Meng, X.Y., Zhang, H.X., Mezei, M. and Cui, M., 2011. Molecular docking: a powerful approach for structure-based drug discovery. Current computer-aided drug design, 7(2), pp.146-157.*
8. *NSW Department of Primary Industries 2007, PRIMEFACT 418, Enterotoxemia in cattle. www.dpi.nsw.gov.au. February 2007*
9. *Songer, J.G., 1996. Clostridial enteric diseases of domestic animals. Clinical microbiology reviews, 9(2), p.216.*
10. *Uzal, F.A., Vidal, J.E., McClane, B.A. and Gurjar, A.A., 2010. Clostridium perfringens toxins involved in mammalian veterinary diseases. The open toxinology journal, 2, p.24.*
11. *Van Metre, D., 2010. Colarado State University Extension, https://extension.colostate.edu/topic-areas/agriculture/enterotoxemia-overeating-disease-of-sheep-and-goats-8-018/* Dated: 25.02.2019.