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In silico assessment of *Nandhi Mezhugu* as a Potential Therapeutic Agent against Diethylnitrosamine (DEN) – Induced Hepatocellular Carcinoma; Molecular Docking Insights

Indumathi. R[1*], Ganne Venkata Sudhakar Rao[2], Pazhanivel. N[3], Srinivasan. M.R.[4], Jalantha. P[4], and Sowmya . M[5]

Department of Veterinary Pathology, Madras Veterinary College, Chennai – 600007, Tamil Nadu.

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Abstract

Hepatocellular carcinoma is the most common liver tumour in dogs. It is the sixth most common cancer in the world in humans and ranks third in causing cancer related mortality. Diethyl nitrosamine (DEN) is a potent liver carcinogen which is ideal for induction of liver cancer in experimental carcinogenesis. Caspase – 3, Bax , BCl2, NF-κB are few of the many tumour markers which are useful in diagnosis of cancer. Nandhi Mezhugu is a Siddha - herbo mineral formulation that has been scientifically validated is being used for rheumatism, skin diseases and various types of cancers. The active principles in Nandhi Mezhugu namely Withaferin-A , Brucine, Piperine, were selected and their structures were retrieved. Molecular docking was performed against 5 tumour marker proteins, Caspase – 3, Bax, , BCl2, NF-κB. The results showed that Withaferin- A had the most favourable binding affinity, followed by Brucine, Piperine . The results of this study indicated a profound *insilico* interaction between the active principles present in Nandhi Mezhugu and the tumour markers.

*Corresponding author : Email : indhuuravii9761@gmail.com
[1]Post Graduate Scholar, Department of Veterinary Pathology, Madras Veterinary College, Chennai -7
[2]Professor and Head, Department of Veterinary Pathology, Madras Veterinary College, Chennai -7
[3]Professor, Department of Veterinary Pathology, Madras Veterinary College, Chennai -7
[4]Assistant Professor, Laboratory Animal Medicine Unit, DCAHS, MMC, Chennai -51
[5]Post Graduate Scholar, Department of Veterinary Pathology, Madras Veterinary College, Chennai -7.

Key words: Hepatocellular carcinoma, Diethylnitrosamine (DEN), Nandhi Mezhugu, *insilico.*

Hepatocellular carcinoma (HCC) is the most common form of liver neoplasia in dogs (Banzato *et al.,*2019). Hepatocellular carcinoma (HCC) or hepatoma includes 90% of all primary liver cancer cases. It is the sixth most common cancer in human in the world and third most common cause of cancer related mortality (Chedid *et al.*, 2017). Diethynitrosamine (DEN), a widely used hepatocarcinogen (Song *et al.*, 2020). DEN is used for inducing liver cancer in rodents as an experimental model of HCC (Anwar *et al.,* 2021).

In cancer, apoptotoic proteins such as Bcl2 are overexpressed, Bax has reduced expression (Su *et al.*, 2013) and there is a lower expression of Caspase -3 (Siddiqui *et al.*, 2017). Inflammatory markers such as NF-κB (Amin *et al* ., 2016) are expressed more.

Nandhi Mezhugu is a polyherbomineral Siddha formulation that has been validated scientifically in the area of safety (Kantham *et al.,* 2017). It has been indicated for rheumatism, skin diseases and various types of cancers in Siddha literature (Sharma *et al.*, 2003). It contains 53 ingredients and most of them have a lot of active principles that may aid in various ailments (Lakshmikantham *et al* .,2017).

In this study the binding affinity of Caspase- 3, Bax, NF-κB , Bcl2 was evaluated against a few active principles present in the ingredients of Nandhi Mezhugu such as Witha-

ferin-A, Brucine, Piperine.

Materials and Methods

Proteins

The 3D protein structures of Caspase- 3, Bax, NF-κB , Bcl2 were downloaded in SDF format and saved in PDB format using Biovia Discovery studio visualizer so as to be used for docking. The protein name and their PDB id are given in Table I.

Table I. Tumour marker and PDB ID

Sl. No.	Tumour Marker	PDB ID
1	Caspase- 3	4JJ8
2	Bax	4S0O
3	Bcl2	1G5M
4	NF-κB	1NFK

Active Principles

The 3D structures of Withaferin, Brucine, Piperine, were downloaded from Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) and PDB websites. The name of the active principle and their IMPPAT and PDB identifiers are given in Table II.

Docking

The selected tumours marker structures as protein targets were converted from PDB to PDBQT formats and kept ready for docking using Autodock Vina (Trott and Olson, 2010). In the same way, ten active principles as ligands were also prepared and saved in PDBQT formats for docking. Each protein was docked individually with the active principle and binding affinity (kcal/mol) was recorded. The adsorbing capacity of the active principle against the tumour marker were assessed by the binding efficiency (ΔG value). Biovia Discovery studio visualizer was used to read the interaction between the tumour markers and active principles present in Nandhi Mezhugu and the output structures were visualized.

Results and Discussion

Molecular formula, molecular weight (g/mol), hydrogen bond acceptor and donor count Log $P_{o/w}$ are given in Table III. The binding affinity (ΔG value) of the tumour marker against each

Table II. Structures of the name of the ingredient, active principles and their respective IMPPAT and PDB IDs

S.No	Ingredient	Active principle	ID
1.	*Withania somnifera*	Withaferin A	IMPHY004118
4.	*Strycnous nux vomica*	Brucine	IMPHY000964
7.	*Piper nigrum*	Piperine	IMPHY004192

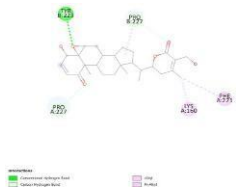
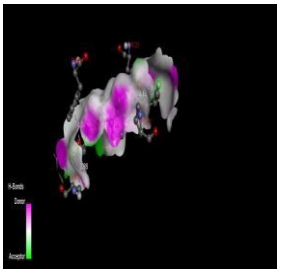
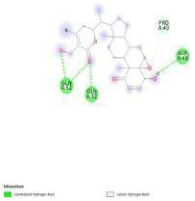
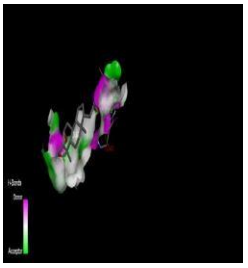
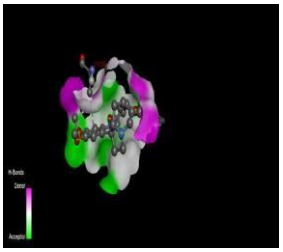
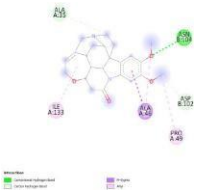
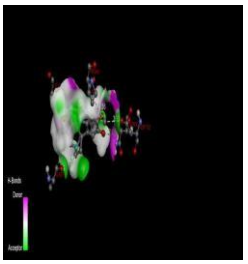
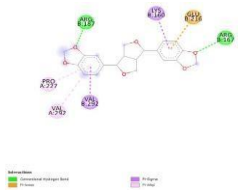
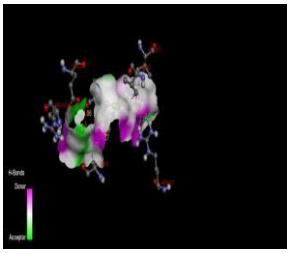
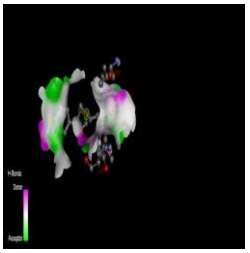
Table III. Chemical nature of active principles

Sl.No.	**Active principles**	**Molecular formula**	**Molecular Weight g/mol**	**Hydrogen Bond Acceptor Count**	**Hydrogen Bond donor Count**	**Log $P_{o/w}$ value**
1.	Withaferin A	C28H38O6	470.60	6	2	3.42
4.	Brucine	C23H26N2O4	394.46	5	0	1.89
7.	Piperine	C17H19NO3	285.34	3	0	3.03

Table IV. Binding affinity between the active principles and tumour markers

Active principles	**Tumour Markers**			
	Caspase-3	**Bax**	**BCl2**	**NFκB**
Withaferin	-10.5± 0.56	-8.7± 0.42	-8.3± 0.37	-8.7± 0.42
Brucine	-7.9± 0.21	-8.0± 0.49	-8.3± 0.33	-7.7± 0.27
Piperine	-9.2± 0.15	-8.1± 0.29	-7.9± 0.21	-8.6± 0.38

Table V. 2D and 3D output structures

Active principles	Caspase- 3		Bax	
	2D	3D	2D	3D
Withaferin-A				
Brucine				
Piperine				

active principle is shown in the Table IV and the 2D and 3D output structures are given in the Table V. Among the active principles, withaferin has greastest binding affinity with Caspase – 3, Bax, BCl2 and NF-κB. Brucine and Piperine had the second highest binding affinity.

Caspases are a class of cysteine proteases that are mainly divided into two groups according to their functions in apoptosis (caspase-3/6/7/8/9) and inflammation (caspase-1/4/5/12). Active caspase-3 degrades multiple cellular proteins and is responsible for morphological changes and DNA fragmentation in cells during apoptosis (Mcllwain *et al*., 2013).

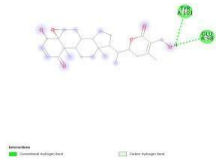
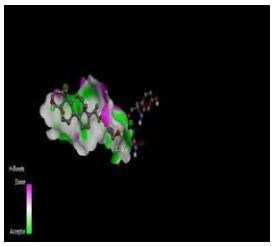
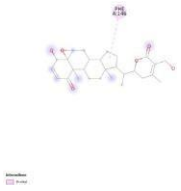
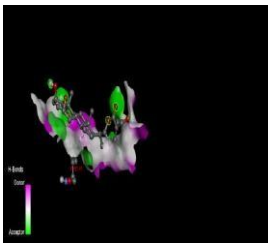
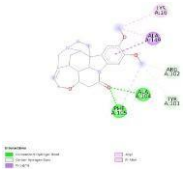
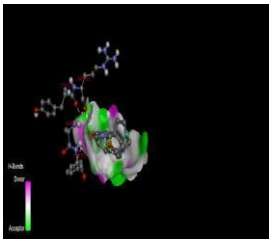
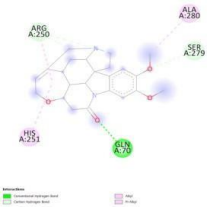
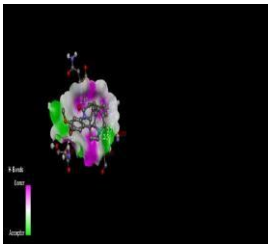
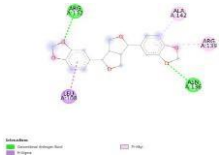
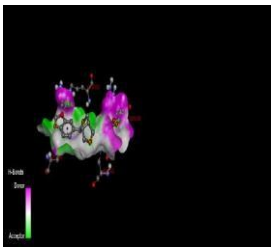
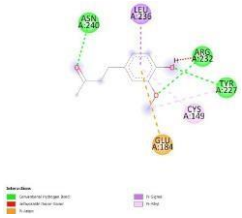
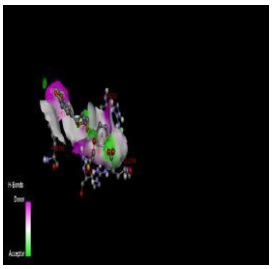
Mitochondrial Outer Membrane Permeabilization (MOMP) and cell death occurs due to pro apoptotic proteins such as Bax (Wei *et al*., 2001) as well as anti apoptotic proteins like Bcl-2, which also participate in fission and fusion of mitochondria (Lucantoni *et al*., 2021). Nuclear Factor kappa B (NF-κB) is a transcriptional factor that helps in cell growth and tumourigenesis (Jana *et al*., 2017).

Withaferin-A produces apoptosis by BCl2 down regulation, mitochondrial translocation and activation of caspase- 3 (Mayola *et al*., 2011). It modulates NF-kB signaling pathway and acts as an anti-inflammatory (Kumar *et al*., 2002).

Brucine and Piperine causes upregulation of Bax and down regulation of BCl2 (Zheng *et al*., 2013), acts as caspase -3 (Kirubhanand *et al*.,2020) and NF-kB inhibitor (Lei *et al*., 2002).

In conclusion, it has been proven in various *invivo* and docking studies that the above mentioned active principles potentially inhibit various markers which involve tumourigenesis. Only a few of the many active principles

Table V contd. 2D and 3D output structures

Active principles	BCl-2		NF-κB	
	2D	3D	2D	3D
Withaferin-A				
Brucine				
Piperine				

of Nandhi Mezhugu have been studied till now. This shows strongly that Nandhi Mezhugu could be used as a treatment for cancer due to its easy availability and affordable cost. Further studies should be carried out to explore more about the hidden beneficial properties present in Nandhi Mezhugu.

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