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In silico anticancer targets of L-menthol

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Abstract

Menthol is a cyclic monoterpene which is used as flouring and therapeutic agent. It has antibacterial, antifungal, and anticancer properties. Many reports confirmed its activity as anticancer molecule. The present work was design to prove its anticancer property *in-vitro* as well. Docking of L-menthol with different cancer protein was carried out to confirm its mode of action on cancer cells. The results are very promising and they directly indicating that the menthol is targeting the proteins which play major role in cancer progression.

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Introduction

Plants extracts and pure molecules isolated from the plants have been considered to be promising agents to treat many diseases (Balunas and Kinghorn, 2007). L-Menthol, a cyclic monoterpene (Fig. 1) present in almost all the different varieties of Mentha (Nair, 2001). The synthesis of L-menthol is also very easy so can be abundantly produced in the laboratory. The uses of L-menthol vary from flavorings agent to antimicrobial agent (Bromm *et al.*, 1995; Kamatou Guy *et al.*, 2013; Sobral *et al.*, 2014). Many reports also confirm its anticancer potential as well In-vitro studies indicate that the L-menthol has multiple targets especially the tubulin (Faridi *et al.*, 2011) and topoisomerase (Jing-Pin *et al.*, 2005).

Microtubules dynamics play crucial role in call division and cell skeleton formation. Many anticancer drugs target the microtubule dynamics which blocks the cells in mitotic phase and finally the cells undergo apoptosis (Jordan *et al.*, 1998; Leroy *et al.*, 2006; Parker *et al.*, 2014), the in-vitro studies indicate that the L-Menthol acts like taxol and disrupts the cell division by modulating the microtubule dynamics (Faridi *et al.*, 2011).

The topoisomerase poisons are well known anticancer agents like etoposide and camptotheacin. These are shown to stimulate DNA cleavage by topoisomerases leading to cell death (Bromm *et al.*, 2002; Leroy *et al.*, 2006). Many *in-vitro* studies shown that L-Menthol decreases the level of topoisomerases I and II, which leads to cell death (Jing-Pin *et al.*, 2005). In one investigation, genes for many BCL-2 family proteins showed higher expression in L-menthol-treated cells (Inohara *et al.*, 1998; Youle *et al.*, 2008).

L-menthol also reported to activate the Tumor necrotic factor surface receptors (TNFSF) and caspase cascade it especially activates caspase-3 and promotes apoptotic process in the cells (Ware, 2008; McIlwain *et al.*, 2013). There are several methods available used in drug development like combinatorial chemistry, de-novo (Yukata *et al.*, 2013), HTS and Computational methods (Hageway *et al.*, 2011). In-silico study is proved to be one of the best methods to validate or analyze the results. In in-silico method, different software programs are used to understand and validate the biological results (*in-vitro* and *in-vivo*). In-silico method became an indispensable tool in drug discovery and development process as it accelerates the process and reduces the cost and time of drug discovery and development process. Molecular docking is among numerous methods used in drug discovery and development process. In molecular docking, the interaction between probable drug molecule (Ligand) and target protein (Receptor) are analyzed by using different docking software (Kazemi *et al.*, 2013; Vishnuvarthan *et al.*, 2017). In present studies Hex 8.0 docking software was used for docking purpose. The objective of the present study was to confirm the plausible anticancer targets of L-menthol. The data from the present study indicates the L-Menthol can bind with tubulin and topoisomerase and other apoptosis-related proteins.

Material and methods

Different bioinformatics tools have been used to carry out the present study. The PubMed Central (PMC), Protein Database (PDB), PubChem and Hex 8.0 were used to carry out experiments and to analyze the data. PubChem was used to get the PDB format of L-menthol (Wang *et al.*, 2009) and Hex 8.0 was used to perform docking. The docking was analyzed by the instructions given in the hex 8.0 manual.

Chem-draw software was used to draw the structure of L-menthol (*Li et al., 2004*). For docking purpose, Hex8.0 was used. Hex is easy to use, freely available and an interactive tool used for the calculation and display of convenient docking mode of pairs of DNA as well as protein. Hex can also calculate receptor and ligand docking, assuming ligand as a rigid body (Vishnuvarthan *et al.*, 2017). Three-dimensional structures of proteins (Tubulin alpha-beta dimer (1TUB), Human Topo IIa(1ZXM), Caspase-3(2XYG), BCL-X complex with BAD (1G5J), TNFSF8(3K48) were retrieved from the Protein Data Bank (PDB)(Fig. 2), a freely available resource. PDB provides all the information regarding various proteins obtained by

X-ray crystallography, NMR etc. (Berman, 2008). The parameters used in the docking process are mentioned in Table 1.

Results

The E-Value of L-menthol with Tubulin alpha-beta dimer, Human Topo IIa, Caspase-3, BCL-X complex with BAD and TNFSF8 are -146, -203.9, -176.21, -188.06 and -221.48 respectively (Table 2 & Figure 3).

The results indicate that all the selected proteins have very good binding affinity especially Topo IIa and TNFSF-8. The following results further approve the *in-vitro* and *in-vivo* finding regarding the mode of action of L-menthol.

Table 1. The parameters used in the docking process.

Correlation type	Shape Only
FTT mode	3D Fast lite
Grid Dimension	0.6
Receptor Range	180
Ligand Range	- 180
Twist Range	- 360
Distance Range	- 40

Table 2. Effect of L-menthol over different targets.

S.No	Target protein	PDB ID	E-value
1.	Tubulin alpha-beta dimer	1TUB	-146
2.	Human Topo IIa	1ZXM	-203.9
3.	Caspase-3	2XYG	-176.21
4.	BCL-X complex with BAD	1G5J	-188.06
5.	TNFSF8	3K48	-221.48

Discussion

For the Structural based drug designing, protein-ligand interaction plays a significant role in drug discovery and development (Wishart *et al.*, 2006). The present study was to establish the in-vitro and in-vivo cancer targets of L-Menthol.

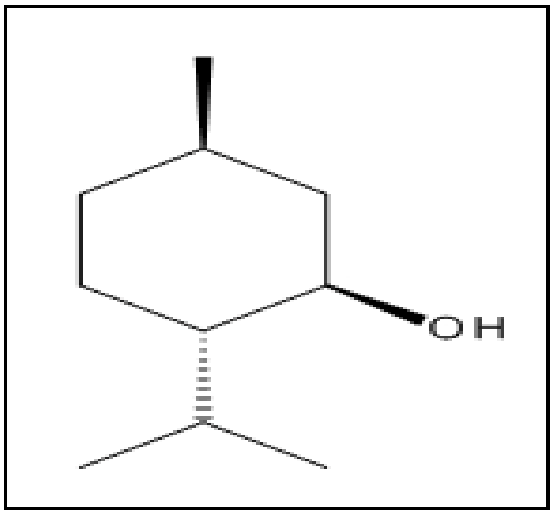

Fig. 1. Structure of L-Menthol.

The role of tubulin dynamics is well established in cell division and many anticancer drugs used tubulin dynamics as an anticancer target. Taxol and Vinca alkaloids are well-known examples.

The previous studies indicate that like taxol, L-Menthol also binds with tubulin proteins and disrupts its dynamics. Topoisomerases inhibitors are also one of the most crucial targets for the cancer therapeutics (Tadashi *et al.*, 2016). One study also shows that L-menthol also binds with topoisomerase II and prevents DNA synthesis in cancer cells. Other apoptosis-related proteins like Tumor necrosis factor surface receptor 8 (TNFSF8), which activates extrinsic apoptotic pathway also interacts with L-menthol. Few studies confirm the role of L-menthol in up-regulation apoptotic proteins like caspase3 and Bcl-2 which play a crucial role in the apoptotic process.

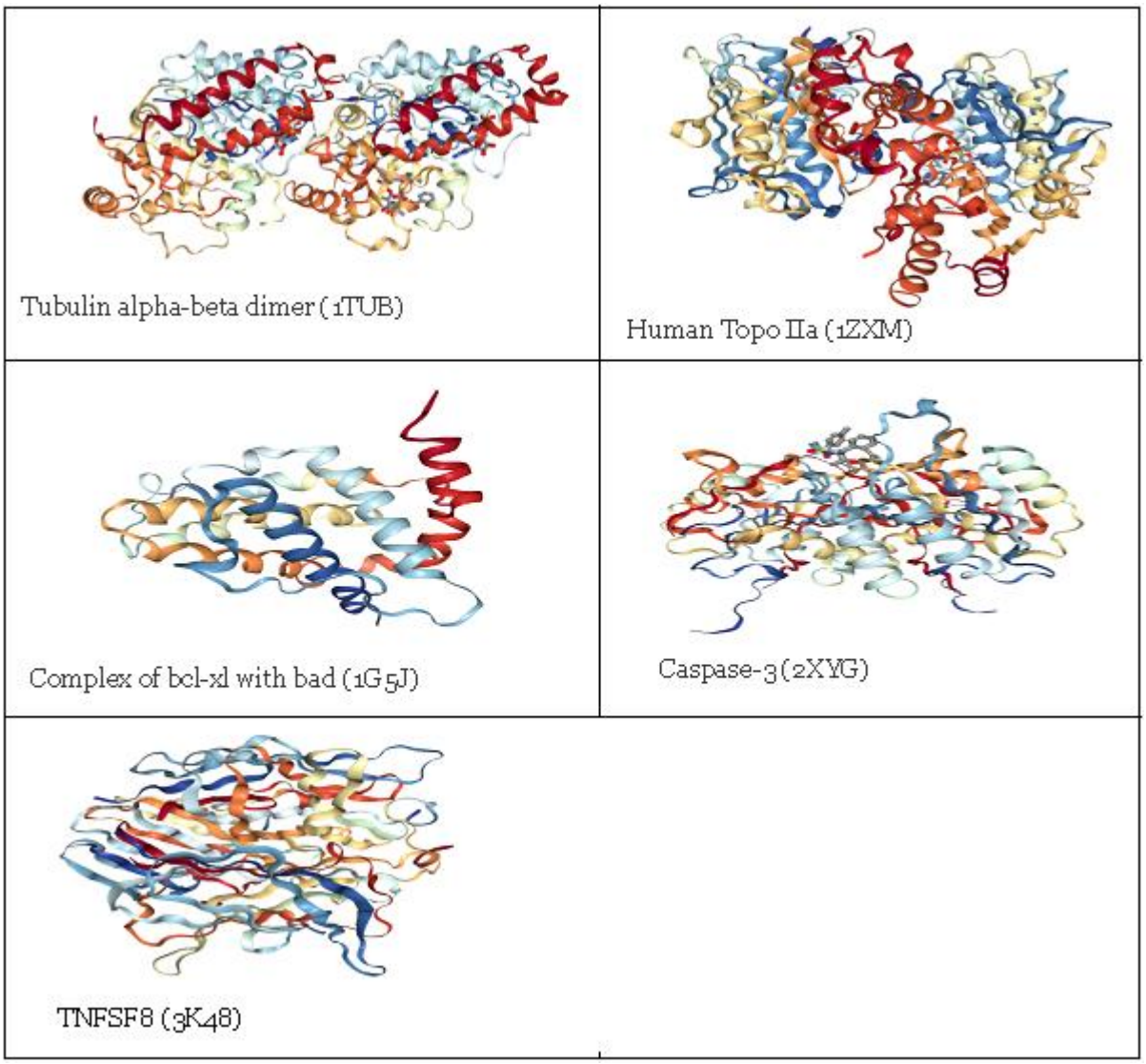

Fig. 2. 3-D Structures of probable targets of L-menthol (adapted from PDB).

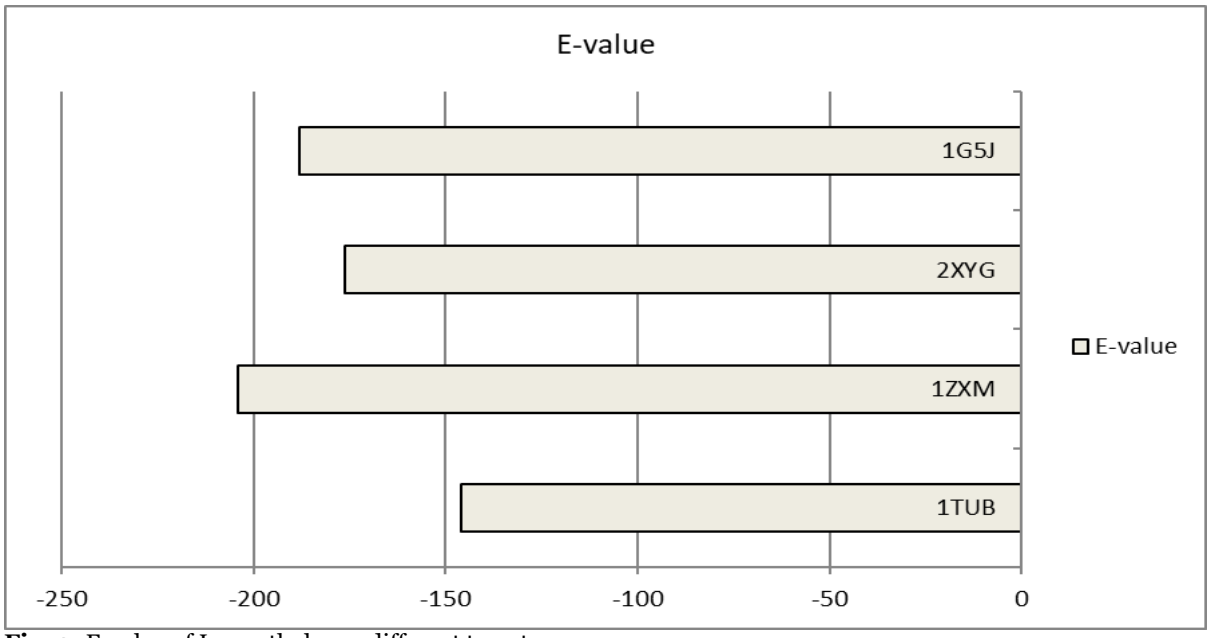

Fig. 3. E-value of L-menthol over different targets.

There are only few molecules which show the multiple cancer targets (Chang Jang-Yang *et al.*, 2003). Our results gave a further insight of L-menthol as an anticancer agent with multiple targets (Table-2) (Figure-3).

Conclusion

The protein and ligand binding plays a very important role in drug discovery. In present study we have selected *in-vitro* and *in-vivo* approved target proteins. Our in-silico studies are confirming them as potent targets.

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