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Appraisal of hypotensive effect of natural monoterpene: Bornyl acetate and *in-silico* molecular docking analysis

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Abstract: The modern trend of research is highly focused on finding new bioactive molecules from medicinal plants. As a functional bicyclic monoterpene, Bornyl acetate (BA) has displayed antioxidant and anti-inflammatory properties in different types of cells and tissues. The purpose of this research was to evaluate the probable hypotensive effect of BA, an underlying mechanism(s) backboned by *in-silico* studies. Mean arterial pressure and heart rate were recorded via invasive blood pressure measuring technique in normotensive Sprague-Dawley rats following the administration of BA (1-80mg/kg). Docking studies were carried out with various targets involved in the pathophysiology of hypertension.RO5 and ADMET properties were also evaluated. In the current study dose-dependent reduction in systolic, diastolic and mean arterial pressure was observed. Pretreatment with atropine and captopril significantly ($p<0.001$) reduced the hypotensive effect produced by BA. On the other hand docking studies showed pronounced interactions with M2 mAch receptor in an agonistic way and ACE protein in an antagonistic way. BA justified all cut-off limits of RO5 and had an acceptable predicted computational toxicity profile. Results postulate that dose-dependent hypotensive effect of BA is mediated through the muscarinic pathway and ACE inhibitory activity corresponding well with findings of *in-silico* studies.

Keywords: Bornyl acetate, invasive technique, hypotensive, molecular docking.

INTRODUCTION

Hypertension is the most quotidian risk factor of cardiovascular and chronic kidney dysfunctions. It is estimated to cause 16.5% annual deaths worldwide. However unfortunately, it remains poorly handled everywhere (Younis *et al*., 2020), with statistics of the worldwide prevalence of adults in 2000 estimated at 26.4% (972 million people, including 639 million in resource-limited countries) and could exceed upto 29% by 2025 (1.56 billion of peoples)(Kpegba *et al*., 2018). Many people in developing countries have limited access to modern healthcare, despite the vast number of antihypertensive medicines available and cannot afford these medications because of the high cost. Pakistan stands among Low-income countries with high prevalence and low awareness about the disease.

Plants have remained historically significant over the last 2 decades as sources of novel compounds with the potential to be considered as lead compounds for future safe, efficacious and cost effective antihypertensive and cardio protective drugs (Iwalokun *et al*., 2011). However it is a nerve wrenching journey of several series of basic research before any bioactive molecule can be consider as a lead compound or its classification as a potential drug. Yet, computer-aided drug discovery and designing (*in-silico* techniques) prove to be very buttressed in pipelining the isolated compounds, phytochemical constituents and related possible ligands on way to an authenticated and potential lead compound. The development of pharmacophore-based molecular docking and scoring techniques are the two important aspects involved in predicting molecular interactions in computer-aided drug design (CADD), referred to as in-silico techniques. Therefore, poor pharmacokinetic profiles (ADME) and potential toxicity can be detected early, along with better target identification, collectively avoiding costly late-stage failure in drug development (Guragossian *et al*., 2016). Thus these computational techniques, like molecular docking study could be a very easy gateway in searching effective drugs of natural origin for many highly concerned ailments such as hypertension before their synthesis and testing in a wet lab.

The most volatile constituent present in various conifer oils and many other oils, including red pine (*Pinus*

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densiflora) and valerian (*Valeriana officinalis*), is bornyl acetate (fig. 1). Bornyl acetate has been used intensively for the chemical and active composition of skin creams, wound care, and natural antiseptic agents as a functional bicyclic monoterpene. Moreover, Bornyl acetate has demonstrated its antioxidant and anti-inflammatory effects in various cell and tissue forms (Yang *et al*., 2018). However little has been known and reported about the probable usefulness of bornyl acetate in the treatment of hypertension. Therefore, this study has been designed to evaluate the hypotensive effect of bornyl acetate in normotensive rats and bridging the results of *in-vivo* experiments with conclusions of *in-silico* experiments. Moreover, Bornyl acetate was also subjected to computational analysis for ADME properties and Lipinski rule of Five (RO5) to predict drug-likeness of the phytochemical.

MATERIALS AND METHODS

Chemicals and drugs

Bornyl acetate, *N*-ω-Nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor), atropine (muscarinic receptor antagonist), hexamethonium bromide (A ganglionic blocker), captopril (angiotensin converting enzyme inhibitor) and propranolol (beta-adrenoceptor antagonist), were obtained from Sigma-Aldrich Chemical Co. St- Louis, MO, USA). All chemicals were of analytical grade. Stock solutions of all the chemicals were prepared with distilled water/normal saline/Tween 80 and the dilutions were freshly on the day of experiments.

Animals used

Sprague-Dawley rats (220-250 g) of a local strain have been used which were supplied standardized pellet feed and water ad libitum at the animal house of the University of Sargodha in precisely controlled environments (12h light/dark period, 25±1°C temperature). In compliance with agreed standards for laboratory animal use and treatment (NIH publication number # 85-23, updated in 1985), animals have been given appropriate housing conditions as well as we strive to reduce animal suffering and the number of animals being used. Recorded approval (No. IAEC/UOS/ 2018/45) has been obtained from the Institutional Animal Ethical Committee of the University of Sargodha before the conduct of all the intended research. Both animals were euthanized with an overdose of thiopental at the end of the tests. (over 40mg /kg, I.V.)

In silico experimentation (Dry Lab)

Lipinski Rule of Five was applied on bornyl acetate by using Molinspiration software (v2013.09).Whereas, ADMET (absorption, distribution, metabolism, elimination and toxicity) computational analysis of understudy phytochemical was done by using ACD/Percepta software (v.14.0.0) (Guragossian *et al*., 2016).

Molecular docking

For docking studies selection of targets is a very crucial step. Different important targets involved in various pathophysiological pathways of hypertension were selected. The crystalline structure of various receptor proteins such as ACE protein, Ca^{2+} Channel, Beta 1 adrenoreceptor protein and M2 mAchR protein was taken from the server using PDB ID entry,1086 (Yu *et al*., 2018), 1T0J (Iman *et al*., 2011), 2ycw (Moukhametzianov *et al*., 2011) and 4MQS (Fish *et al*., 2017) respectively.

Using Molecular Operating Environment (MOE) version 2016.11, Chemical Computing Group, all the computational studies were conducted. The software was installed under the 'Windows Server 2003 R2' operating system on an IBM System x3400 with four 3.00 GHz Intel (R) Xeon (TM) CPUs and 2048 Ram processors. The bornyl acetate structure was built using the MOE program's Builder Interface. Using Force field MMFF94x, the structure was then minimized by energy and saved in the db file format. The docking study protocol as explained by Gul *et al*., 2019 was followed. In order to identify the type of interactions which were involved in binding the test compound in the active site of proteins and 2D ligand plots, ligand-protein interaction diagrams of best docked conformation were generated which showed the major amino acids involved in the binding.

In-vivo Experimentation: (WET LAB)

Evaluation of hypotensive effect of bornyl acetate in normotensive rat by invasive technique

The animals were anesthetized with sodium thiopental intraperitoneal injection (70-90mg/kg body weight). To avoid airway obstruction, the trachea was exposed and cannulated with PE 20 tubing. With PE-50 tubing, the left jugular vein and right carotid artery were cannulated. The former was used for the administration of standard drugs and Bornyl acetate whereas the right carotid artery, connected to a pressure transducer coupled to Power Lab recording system with an application program (Chart, v 6.1; all from ADI Instruments; Castle Hill, Australia) was used for blood pressure (BP) and heart rate (HR) measurement. Once cannulated, the animals were allowed to be stabilized for 30 min and then the dose-response relationship to BA (1mg-80mg/kg) was established intravenously, followed by flushing with 0.1ml of saline. Each dose was separated by 10min intervals before the injection of the next dose. In a group of rats, regular saline and 3% Tween 80 solution was also administered as a vehicle to ensure that the reported effects were not due to the action of the vehicle. The difference between the steady-state value before and the lowest reading after the extract injection was recognized as changes in BP (Younis *et al*., 2020).

Appraisal of a possible mechanism of action for the hypotensive effect of bornyl acetate

Animals were anesthetized and subjected to blood pressure measurement following the aforementioned procedure for the elucidation of underlying mechanisms of the hypotensive effect of BA based on the outcomes of docking studies.For this purpose, various agonists and antagonists such as atropine (1 mg/kg), propranolol (1mg /kg), NG-nitro-L-arginine methyl ester (L-NAME, 20mg/kg), captopril 2.5mg/kg and hexamethonium bromide 30mg/kg were used. Each standard drug was given 10min prior to a bolus injection of BA (40mg/kg; which is a representative dose that produced 40-50% of reduction in blood pressure) (Younis *et al*., 2020). Change in SBP and HR shown by intravenous injection of plant extract was evaluated for 40 minutes after treatment.

STATISTICAL ANALYSIS

Data obtained from all *in-vivo* experiments in the current study was expressed as mean ± S.E.M. Using one-way ANOVA accompanied by Dunnett's post-test, findings were statistically analyzed. P values less than 0.05 were considered significant. For statistical analysis, GraphPad prism 5.0 was used.

RESULTS

In-silico experimentation

The Lipinski rule of five was applied to BA and it justified all the cut-off limits of RO5, thus compound possesses drug-likeness and can be a potential lead compound (table 1). Whereas computational analysis of ADMET properties showed that the compound neither has water solubility (as also evident during *in-vivo* dilution preparations), nor has CNS permeability with a very less significant toxicity profile.

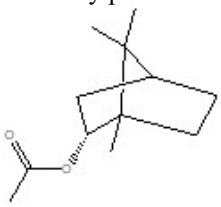
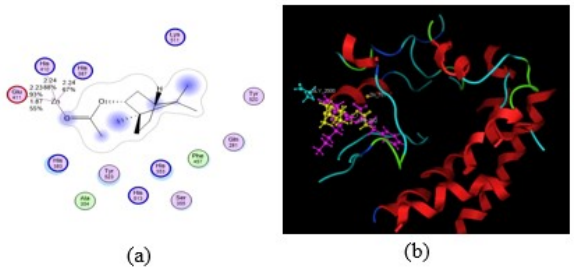


Fig. 1: 2D structure of Bornyl Acetate



(a) (b)

Fig. 2: (a) representing 2D diagram of BA docked with ACE protein showing amino acid and hydrogen bonding involved in the interaction (b) 3D diagram of BA(yellow color ball and stick structure) exactly occupying bound lisinopril ligand (Purple colored ball and stick structure) in the active site of ACE protein (PDB ID 1O86).

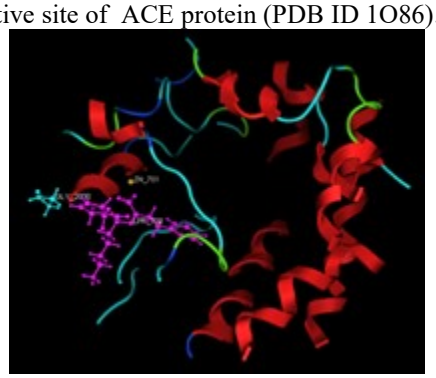
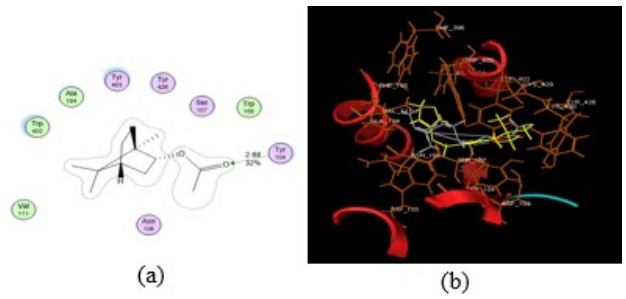


Fig. 3: Fig showing the crystal structure of ACE protein with bound lisinopril ligand in the active site (PDB ID 1O86).



(a) (b)

Fig. 4: (a) representing 2D diagram of BA docked with M2 mAchR protein showing amino acid and hydrogen bonding involved in the interaction (b) 3D diagram of BA (grey colour line structure) exactly occupying bound ligand (yellow-colored line structure) in the active site of M2 mAchR (PDB ID 4MQS)

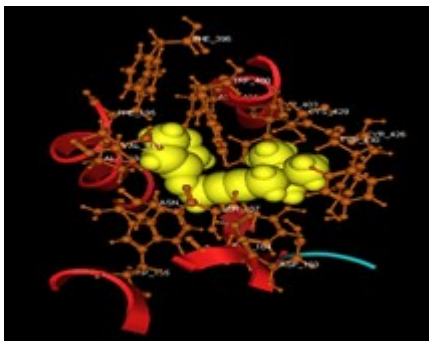


Fig. 5: Fig showing the crystal structure of M2 mAchR with bound ligand in the active site (PDB ID 4MQS)

Molecular Docking

The isolated phytochemical was docked against various targets involved in hypertension pathophysiology out of which it showed well-co-ordinated interactions with ACE-protein and M2 AchR. The compound depicted a strong hydrogen bond with Zn+ in the active site of ACE protein with His410, His387 and Glu411 as involved amino acids (fig. 2a and fig. 2b) similar to that of bound lisinopril ligand in the co-crystal structure of an ACE

Table 1: Table depicting computational analysis of RO5 of BA

Phytochemical constituents	Studied Parameters						
	clog P	Mol.Wt	Rotatable Bonds	Acceptor H-Bonds	Donor H-Bonds	TPSA (Å)	Drug Likeness
Bornyl Acetate	2.76	196.29	2	2	0	26.30	YES

Table 2: Dose dependent decrease in MSBP, MDBP and MABP of BA in anesthized rats

Parameters	CONTROL	1MG	5MG	10MG	20MG	40MG	80MG
MSBP	128.70±3.48	120.01±2.22 (6.7%)	118.54±2.26 (7.8%)	105.79±7.71 (17.80%)	**74.57±14.60 (42.05%)	**66.55±14.85 (48.29%)	***40.51±5.33 (68.52%)
MDBP	118.10±1.63	111.67±3.86 (5.4%)	109.98±3.37 (6.8%)	99.75±8.55 (15.5%)	**60.38±14.99 (48.87%)	**58.16±16.09 (50.75%)	***32.63±5.03 (72.37%)
MABP	122.65±1.74	115.18±2.62 (6.09%)	111.91±3.68 (8.7%)	101.92±8.17 (16.90%)	**69.93±16.98 (42.98%)	**60.59±15.59 (50.5%)	***35.79±5.30 (70.8%)

protein (PDB ID 1086) (fig. 3).On a similar basis, BA illustrates wander wall bonding with M2 mAchR with involved amino acids as Tyr104, Tyr 403, Tyr 426, Asn108, Val111, Trp400 and Ala194 (fig. 4a and fig. 4b) similar to that of a bound ligand in the crystal structure of M2 mAchR (PDB ID 4MQS) (fig. 5).

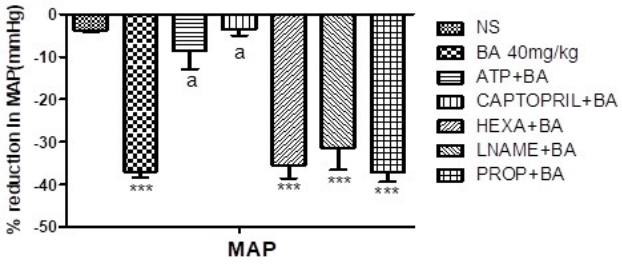


Fig. 6: Figure illustrating effect of various agonists and antagonists on hypotensive effect of BA (40mg/kg). Results are indicated as mean ± S.E.M. where *** =p<0.001 when compared to control (Normal saline treated group) while a = p<0.001 when compared to treated control (BA, 40 mg/kg). All data are subjected to one-way ANOVA followed by Dunnett's posttest).Where ATP= atropine, PROP= propranolol and HEXA= hexamethonium,

In-vivo experimentation

Acute hypotensive effect of bornyl acetate

After stabilization period of 30 minutes, the basal SBP observed in the anesthetized control rats was 128.70± 3.438 mm Hg. There was no impact of the intravenous administration of the vehicle on blood pressure and heart rate (data not shown). BA administration resulted in a substantial and rapid decrease in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure, respectively (MBP). The dose-dependent decrease in SBP was caused by the intravenous administration of BA (1, 5, 10, 20, 40 and 80mg/kg). (table 2) with percentage reduction as 6.7%, 7.8%, 17.80%, 42.05%, 48.29% and 68.52% from 1mg to 80mg/kg respectively, with minor effects on heart rate (data not shown).

Possible mechanism underlying hypotensive effect of bornyl acetate

As predicted by the *in-silico* studies prior administration of captopril and atropine attenuated the decrease in MAP produced by BA in normotensive rats. Where as for a counter check administration of L-NAME, hexa-methonium and propranolol did not produced any significant effect on the magnitude of decrease in MAP produced by BA (fig. 6).

DISCUSSION

Cellular oxidative stress and inflammation are closely related to cardiovascular diseases (CVDs) (Ooi et al., 2018). The terpenes is the significant class of secondary plant metabolites having various pharmacological characteristics such as antioxidants, hypotensive and vasorelaxant. Thus these terpenes may be useful as agents for cardiovascular diseases prevention and/or treatment, including hypertension, (CVD) (Santos *et al*., 2011). The results of our present study correspond very well with these finding as BA produces dose dependent hypotensive effect in anesthetized normotensive rats.

Further results about the probable mechanism of action that might be involved in the hypotensive effect produced by BA clearly depicted a decrease in the hypotensive effect of BA in presence of atropine; an muscarinic antagonist and Captopril (an ACE inhibitor). Atropine may act by blocking the acetylcholine effect on the heart. The activity of endothelial acetylcholine acting on the M3 receptor can also be blocked by atropine, which causes vasodilation. These promising results correspond very well with our finding through molecular docking which showed very pronounced interaction of BA with the computational M2 mAch receptor (fig. 4a and fig. 4b), thus strengthens our *in-vivo* experimentation findings that BA might act through binding to muscarinic receptors producing action similar to that of Acetylcholine.

Similarly pretreatment with captopril also prominently decreased the magnitude of hypotension produced by BA

(40mg/kg) which depicts that BA also act by following RAAS pathway by inhibiting ACE enzyme thus blocking the production of angiotensin II (a potent vasoconstrictor). Our findings are again backbone by docking studies which showed notable interaction of BA with ACE enzyme by involving Zn(II) . The involvement of Zn(II) in the active centre of the enzyme (fig. 2a and fig. 2b) played a critical role in the activity of the ACE inhibitor, as ACE amino acid residues His410, Glu411, His387 formed coordination bonds with the prothetic group Zn(II). This can explain the deformation of the tetrahedral coordination of Zn(II) and accelerated ACE deactivation (Yu *et al*., 2018). Thus reinforcing the facts of in-vivo experimentation findings that BA might be a potential ACE inhibitor.

CONCLUSION

In conclusion, we present that the natural monoterpene bornyl acetate has a prominent hypotensive effect and it might be speculating this effect through muscarinic receptors and possible ACE inhibitor activity, the results which also correlate very well with findings of molecular docking studies. Thus this study signifies the therapeutic role of natural terpenes in the treatment of CVDs particularly hypertension and paves way for many horizons for the research fraternity for the development of BA as a useful lead compound.

REFERENCES

Akhtar MS, Bashir S, Malik MH and Manzoor R (2013). Cardiotonic activity of methanolic extract of Saussurealappa Linn roots. *Pak. J. Pharm. Sci.*, **26**(6): 1197-1201.

Fish I, Stoßel A, Eitel K, Valant C, Albold S, Huebner H, Mooller D, Clark MJ, Sunahara RK, Christopoulos A and Shoichet BK (2017). Structure-based design and discovery of new M2 receptor agonists. *J. Med. Chem.*, **60**(22): 9239-9250.

Gul A, Akhter Z, Perveen F, Kalsoom S, Ansari FL and Siddiq M (2019). Molecular docking and quantitative structure activity relationship (QSAR) studies of some newly synthesized poly (azomethine) esters. *Int. J. Polym. Sci.*, pp.1-10

Guragossian N, Gozzi GJ, Fouillet B, Gustin MP, Terreux R, Bouaziz Z, Marminon C, Jose J, Di Pietro A, Pasanen M and Le Borgne M (2016). Candidate molecule selection based on *in silico* predicted ADMET properties of 12 indenoindole derivatives. *Chem. Informatics.* 2: 1-10

Iman M, Davood A, Nematollahi AR, Dehpoor AR and Shafiee A (2011). Design and synthesis of new 1, 4-dihydropyridines containing 4 (5)-chloro-5 (4)-imidazolyl substituent as a novel calcium channel blocker. *Arch. Pharm. Res.,* **34**(9): 1417.

Iwalokun BA, Hodonu SA, Nwoke S, Ojo O and Agomo PU (2011). Evaluation of the possible mechanisms of antihypertensive activity of *Loranthus micranthus*: An African mistletoe. *Biochem. Res. Int.,* pp.657.

Kpegba K, Tete Kondo E, Simalou O, Togbenou K, Boyode P, Toundou O, Gbandjaba Y, Agbonon A and Gbeassor M (2018). A significant antihypertensive effect of Holarrhena floribunda supported by an exploratory phytochemical study. *J. Herb. Med. Pharmacol.,* **7**(3): 160-167.

Moukhametzianov R, Warne T, Edwards PC, Serrano-Vega MJ, Leslie AG, Tate CG and Schertler GF (2011). Two distinct conformations of helix 6 observed in antagonist-bound structures of a β1-adrenergic receptor. *PNAS*, **108**(20): 8228-8232.

Ooi BK, Chan KG, Goh BH and Yap WH (2018). The role of natural products in targeting cardiovascular diseases via Nrf2 pathway: Novel molecular mechanisms and therapeutic approaches. *Front Pharmacol.,* **9**: 1308.

Santos MR, Moreira FV, Fraga BP, Souza DPD, Bonjardim LR and Quintans-Junior LJ (2011). Cardiovascular effects of monoterpenes: A review. *Revista Rev. Brash. Farmacogn.,* **21**(4): 764-771.

Yang L, Liu J, Li Y and Qi G (2018). Bornyl acetate suppresses ox-LDL-induced attachment of THP-1 monocytes to endothelial cells. *Biomed. Pharmacother*., **103**: 234-239.

Younis W, Schini-Kerth VB, da Silva DB, Junior AG, Bukhari IA and Assiri AM (2020). Role of the NO/cGMP pathway and renin-angiotensin system in the hypotensive and diuretic effects of aqueous soluble fraction from *Crataegus songarica* K. Koch. *J. Ethnopharmacol.,* **249**(1): 112400.

Yu F, Zhang Z, Luo L, Zhu J, Huang F, Yang Z, Tang Y and Ding G (2018). Identification and molecular docking study of a novel angiotensin-I converting enzyme inhibitory peptide derived from enzymatic hydrolysates of *Cyclina sinensis*. *Mar. Drugs*, **16**(11): 411.