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Original article

In silico authentication of amygdalin as a potent anticancer compound in the bitter kernels of family Rosaceae

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ABSTRACT

Amygdalin a naturally occurring compound, predominantly in the bitter kernels of apricot, almond, apple and other members of Rosaceae family. Though, amygdalin is used as an alternative therapy to treat various types of cancer but its role in cancer pathways has rarely been explored yet. Therefore, present study was intended with the aim to investigate the alleged anti-cancerous effects of amygdalin specifically on PI3K–AKT–mTOR and Ras pathways of cancer in human body. Computational modelling and simulation techniques were used to assess the effect of amygdalin on PI3K-AKT-mTOR and Ras pathways using different level of dosage. It was observed that amygdalin had direct and substantial contribution to regulate PI3K-mTOR activities on threshold levels while the other caner pathways were effected indirectly. Consequently, amygdalin is a down-regulator of a cancer within a specified amount and contribute considerably to reduce various types of cancer in human. Furthermore, *in-vitro* and *in-vivo* analyses of amygdalin could be of helpful to authenticate its pharmacological effects.

1. Introduction

It is well established that naturally occurring compounds are less toxic than synthetic ones (Nisar et al., 2018; Koparde et al., 2019), and because of this plants and animals based natural products are attaining more attention (Chen et al., 2019). Chinese herbal drug "Taoren (*Semen Persicae*)" contains a primary monomer component i.e. amygdalin (Tanaka et al., 2014). In traditional Chinese medicines, this plant is used to treat asthma, bronchitis, emphysema, leprosy, colorectal cancer, leucoderma, coagulation, anti-inflammatory, as analgesic, neoplastic, thirst-quenching, antipyretic, antitussive as well as to improve microcirculatory disturbance effects (Zhang et al., 2018; Yang et al., 2014; Hwang et al., 2008; Chang et al., 2005).

Amygdalin, which is also known as vitamin B17 or Laetrile (Lv et al., 2017), is an aromatic cyanogenic compound that belongs to sub-class of carbohydrates and carbohydrate conjugates. Chemical formula of amygdalin is $C_{20}H_{27}NO_{11,}$ having molecular weight of 457.432 g/mol and D-mandelonitrile-beta-D-gentiobioside structure (Chang et al., 2006), as shown in Fig. 1. Structure of amygdalin is comprises of two molecules of glucose viz. benzaldehyde and hydrocyanic acid (Shim et al., 2000; Fukuda et al., 2003). Amygdalin is a cyanogenic glycoside, abundantly present in in the kernels of the various species of Rosaceae family such in the bitter seeds of apricot, apple, almond, peaches, cherries, plums, grains, millets, sprouts and nuts (Lv et al., Bolarinwa et al., 2014, 2017; Xu & Song, 2014; Hwang et al., 2008). It has been reported that

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[1] First two authors' have equal contribution in research work and shares first authorship.

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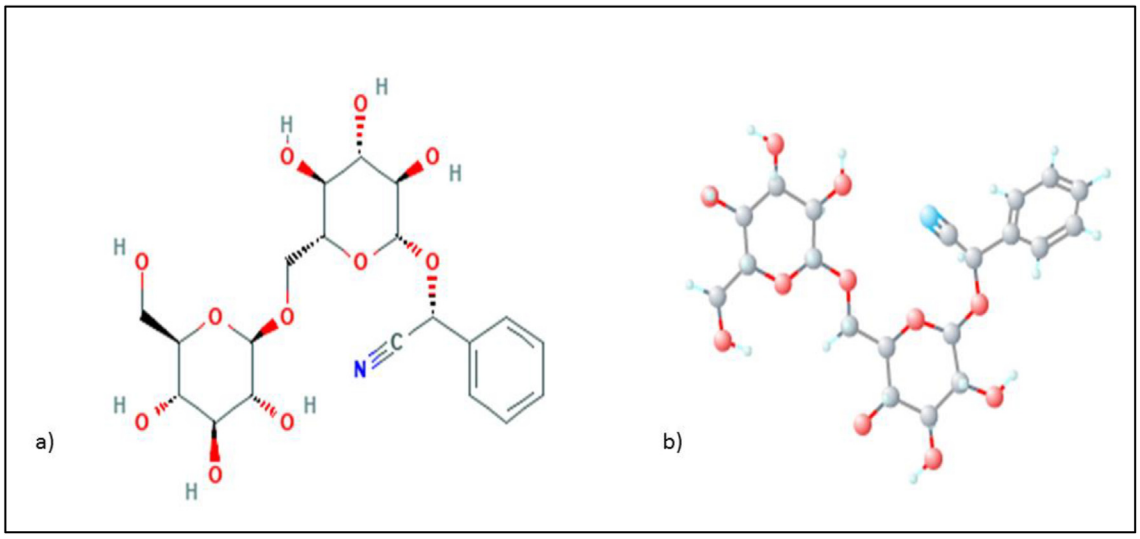

Fig. 1. 2D structure (a) and 3D structure (b) of amygdalin (pubchem.ncbi.nlm.nih.gov).

seeds of Rosaceae fruits contain more amygdalin than endocarps and mesocarps (Lee et al., 2017).

Laetrile which is a semi-conservative form of amygdalin was used as an anticancer agent in 1970s (Integrative, 2017). However, in the late 1970s and early 1980s, after clinically examination Laetrile was rejected by FDA for cancer treatment due to scarce clinically data evidence about its efficacy and toxicity (Moertel et al., 1982). However, it has been used in many parts of the world even FDA refusal (Song & Xu, 2014). In 1987, USA banned the import of amygdalin (Laetrile) in the country, afterwards its use was also banned in Europe (Curran, 1980), but its analgesic, anti-coagulation, anti-neoplastic, anti-inflammatory as well as micro-circulatory disturbance effects were reported. Numerous claims about amygdalin toxicity and beneficial effects were stated, including the treatment of cancer but these claims are not substantial to make the decision due to limited data availability (Saleem et al., 2018; Xu & Song, 2014; Moertel et al., 1981; Dorr & Paxinos, 1978; Ellison et al., 1978). In this context, present study was focused to analyze the effect of amygdalin on a PI3K–AKT–mTOR and Ras pathways in human. Diverse oncogenes and growth factor receptors stimulate the activity of PI3K and elevated PI3K signaling is considered a hallmark of cancer. AKT substrates are involved in cell proliferation, metabolism, survival and motility while the activation of mTOR, specifically mTORC1, is a key concern of tumor-associated alterations that even alter PI3K pathway. Homeostatically balance of PI3K-AKT-mTOR network is critical to prevent the abnormal cellular proliferation (Fruman et al., 2017). Moreover, RAS signaling pathways are the key regulators of several aspects of normal cell growth and malignant transformation (Downward, 2003). In Silico modeling and simulation techniques which are widely adopted for drug developmental process (Deisboeck et al., 2009; Hosseini et al., 2018) were used to determine the activity of amygdalin in up-regulation or down-regulation of cancer pathways.

2. Material and methods

2.1. Selection of drug candidate

Amygdalin was selected as a potential drug candidate to be delivered because of its traditional use for the treatment of many diseases, particularly to treat cancer. This compound is considered as a therapeutics in many parts of the world but there are a lot of conflicts about its cyanide poisoning. Therefore, available literature is not significant enough to make the confirmatory statement about its anti-cancerous efficacy.

2.2. Drug data collection and toxicity assessment

In depth literature survey and Pubchem (https://pubchem.ncbi.nlm.nih.gov) were used for drug data collection. ProTox (http://tox.charite.de/protox_II/) was used for toxicity assessment and lethal dosage (LD50) prediction of drug (Amygdalin). The toxicity class ranges from 1 to 6, those which lie from 1 to 3 have high rates of toxicity while 4 to 6 are acceptable (Drwal et al., 2014).

2.3. Pathway designing

To analyze the effects of amygdalin on cancer, a pathway having multiple nodes such as TOR (target of rapamycin), PI3K (Phosphatidylinositol 3-kinase), RAS (belongs to a class of protein called small GTPase, which involved in transmitting signals) pathways were designed computationally using MATLAB.

MATLAB a computational software was used to design a pathway (Fig. 2), having multiple nodes, which are involved in the abnormal regulation leading to cancer (Rozengurt et al., 2014). The computational demonstration can precisely construct and explain the complete observable facts. The constructed technical model defines the biochemical relationship between different nodes (genes) and the effect of the drug (amygdalin) in different time units. Specifically, mTORC1 functions as a catalytic subunit, to control cellular growth, translation, transcription, as well as autophagy and is the best inhibitor used in the cancer therapy (Sulaimanov et al., 2017; Liu et al., 2016; Laplante & Sabatini, 2012). Moreover, abnormal activation of PI3K, AKT, Ras, Raf, ERK are already reported in multiple human cancers and are evoked to be an attractive therapeutic targets in a range of malignancies (Rozengurt et al., 2014).

2.4. Species data collection

Assigned parameters of the species (proteins) required for simulation such as concentration, amount and molecular weight, were retrieved by online computational search engine ProtParam (https://web.expasy.org/protparam/). Reaction values i.e. molecular weight of species and species reactions, and reaction properties

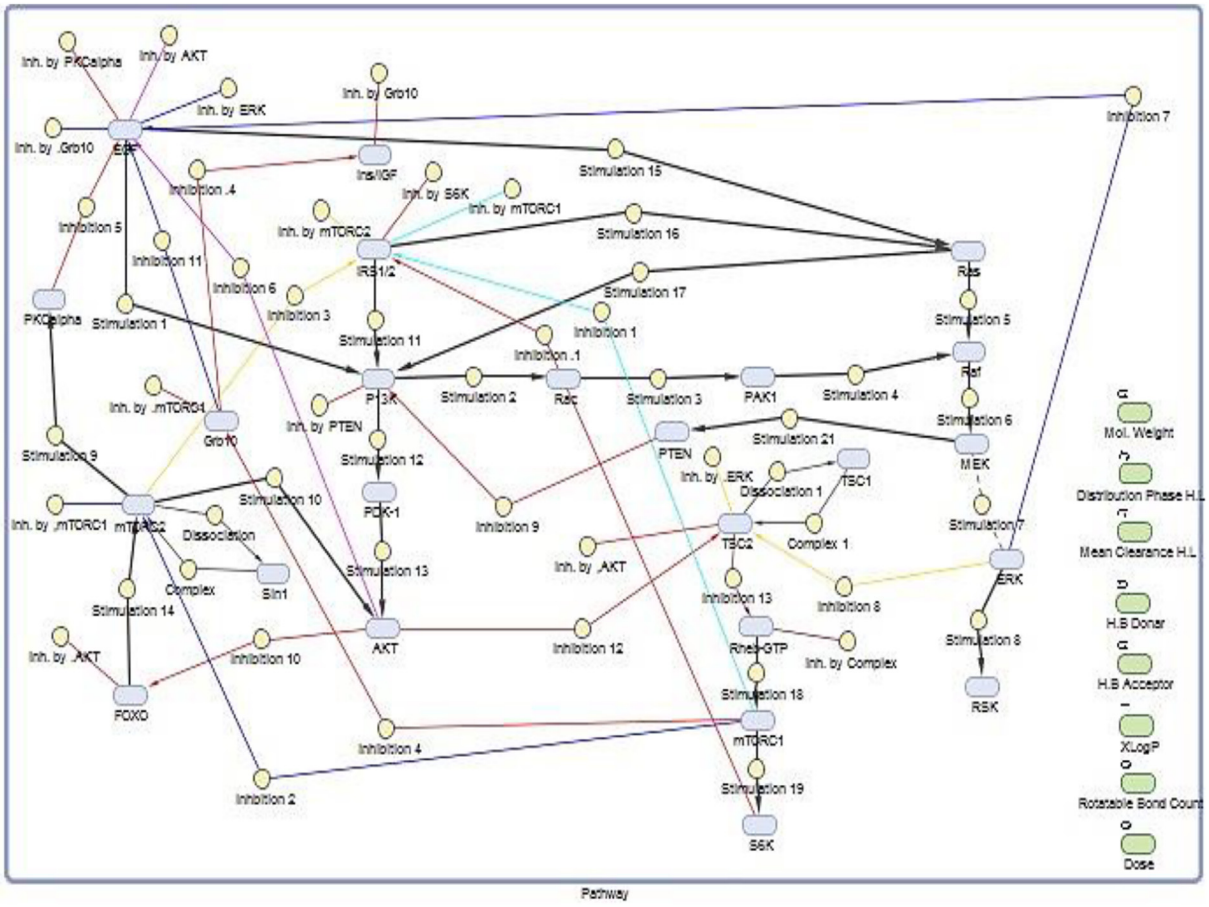

Fig. 2. Pathways designed on MATLAB having 19 stimulatory and 12 inhibitory reactions.

like reaction name, quantities of species used by a reaction and whether reaction causes stimulation or inhibition (kon/koff) were assigned, as reported previously (Rozengurt et al., 2014). The specie properties were assigned as a variants having molecular weights. The minimum and maximum values were assigned automatically by the software, according to the designed pathways.

2.5. Drug properties and dose

Pubchem and ProtParam databases were used as a search engine for collection of the drug properties. The drug properties were assigned in a model parameter having molecular weight distribution phase mean clearance phase hydrogen bond donor hydrogen bond acceptor rotatable bonds and xLogP.

2.6. Simulation

A computational model was developed, which mimics the in vivo simulations. Furthermore, we explain the robustness for systemic modulating behavior of amygdalin during the course of different time unit simulation. Time as well as quantities were adjusted in the add task menu and then model simulations were executed. Analysis was done on the model by adjusting different model conditions as well as comparing the model simulation before and after drug dosage to analyze whether the drug causes up regulation or down regulation of cancer.

3. Results and discussion

Because of proficient outcomes, time saving and cost effective properties, computational (in silico) techniques have attained more consideration in recent years. Over the last decade, these techniques are contributing significantly in PK/PD modeling, hypothesis prediction, drug analysis and for generation of biological model and their testing prior to in vivo and clinical studies (Pichardo-Almarza & Diaz-Zuccarini, 2016; Ekins et al., 2007). In past over 20% drugs were rejected because of toxicity but now computational analysis is making it easy for predicting the toxicity of drug candidates. Same is in the case of amygdalin, which is a used to treat different types of cancer but at the same time it is considered toxic to human and banned in some countries like US and UK. Moreover, in silico analysis of this compound has rarely be done. Therefore, present study was focused on the toxicity assessment and role of amygdalin on PI3K–AKT–mTOR and Ras cancer pathways in human as shown in Fig. 2.

Results of drug properties and relevant information as reported previously (Ames et al., 1981) are given in Table 1. Toxicity analysis done by ProTox revealed that oral toxicity of drug (amygdalin) lied in a class 4 while its predicted LD50 was 405 mg/kg (Fig. 3). The toxicity class ranges from 1 to 6, those which lie from 1 to 3 have high rates of toxicity while 4 to 6 are acceptable (Drwal et al., 2014).

The specie properties were assigned as a variants having molecular weights (Fig. 4). The minimum and maximum values were assigned automatically according to the designed pathway.

Table 1
Drug (amygdalin) parameters.

Parameters	Values
Molecular weight	457.4 g/mol
Distribution phase half life	6.20 min
Mean clearance phase	99.3 mL/min
Hydrogen bond donor	7
Hydrogen bond acceptor	12
Rotatable bonds	7
XLogP	−2.7

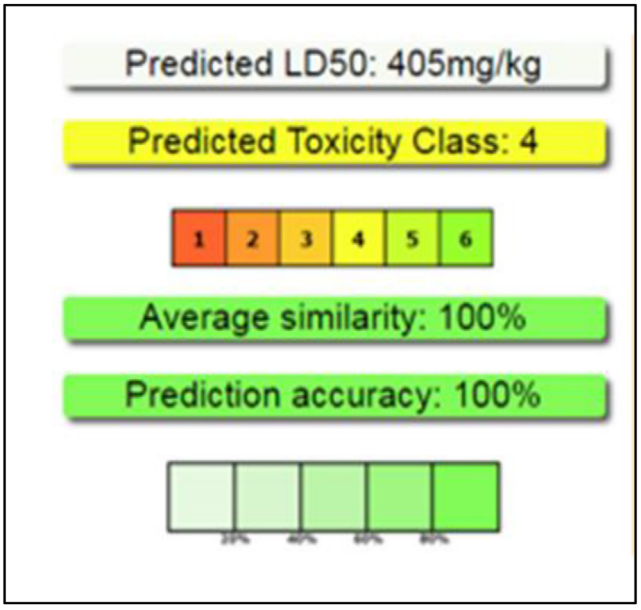

Fig. 3. Oral toxicity of amygdalin http://tox.charite.de/protox_II/).

Pathways designed in simbio module of MATLAB (Rozengurt et al., 2014), having 12 inhibitory and 19 stimulatory reactions were interconnected to each other. Due of its robust properties, integrated pathways help minimize the effects of external perturbation on entire systems. At given rate kinetics, each functional species in the pathways is considered as node and linked to other important nodes (Lehar et al., 2008). Simulation task was applied to analyze the behavior of amygdalin on multiple cancerous nodes. Simulation analyses, which can be used to compare the results of high-throughput experiments, can be carried out with the exploration of the completed reaction plots. When comparing the experimental system with the significance of the results of the pathway model simulation, high validity of the overall mechanism prediction is produced. The actual biological process occurring inside cells can be observed and exploited to predict the individual organism's novel properties and capabilities using this valid model (Chong et al., 2014). The simulation graphs of all the species which were involved in a designed pathway are given in Fig. 5.

To analyze the exact behavior of PI3K, AKT, mTOR and Ras nodes, distinct stimulatory tasks were applied. According to Hill et al., 2014; Thorpe et al., 2015, up-regulation of PI3K occurs during cancer, and we observed that threshold value of amygdalin (250 mL), worked effectively to down-regulate the PI3K. In cancer pathway, the observed maximum concentration of PI3K is $\sim 5.1 \times 10^4$ mL and minimum is $\sim 3.3 \times 10^4$ mL (Fig. 6a). After applying of the amygdalin dose, a decrease in the concentration of PI3K was observed within 12hr, which almost reached to the concentration of $\sim 1.0 \times 10^4$ within 10 days (Fig. 6b).

The mTOR deregulation causes pathophysiological conditions involving aging, Alzheimer's disease, diabetes, obesity and cancer (Hua et al., 2019). The mTORC1 involves in the protein synthesis, cell mass increase, lipid accumulation and cellular energy (Zoncu et al., 2011). In the study model, mTORC1 concentration was $\sim 0.9 \times 10^4$ per day when no dose of amygdalin was applied, and was decreasing progressively. And within 10 days the concentra-

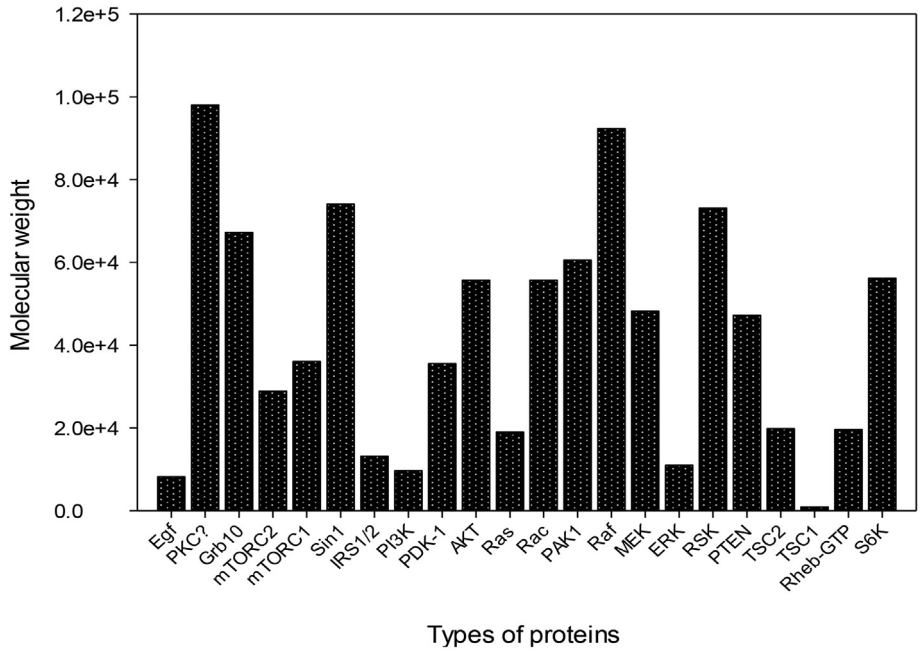

Fig. 4. Molecular weight of the species (Proteins).

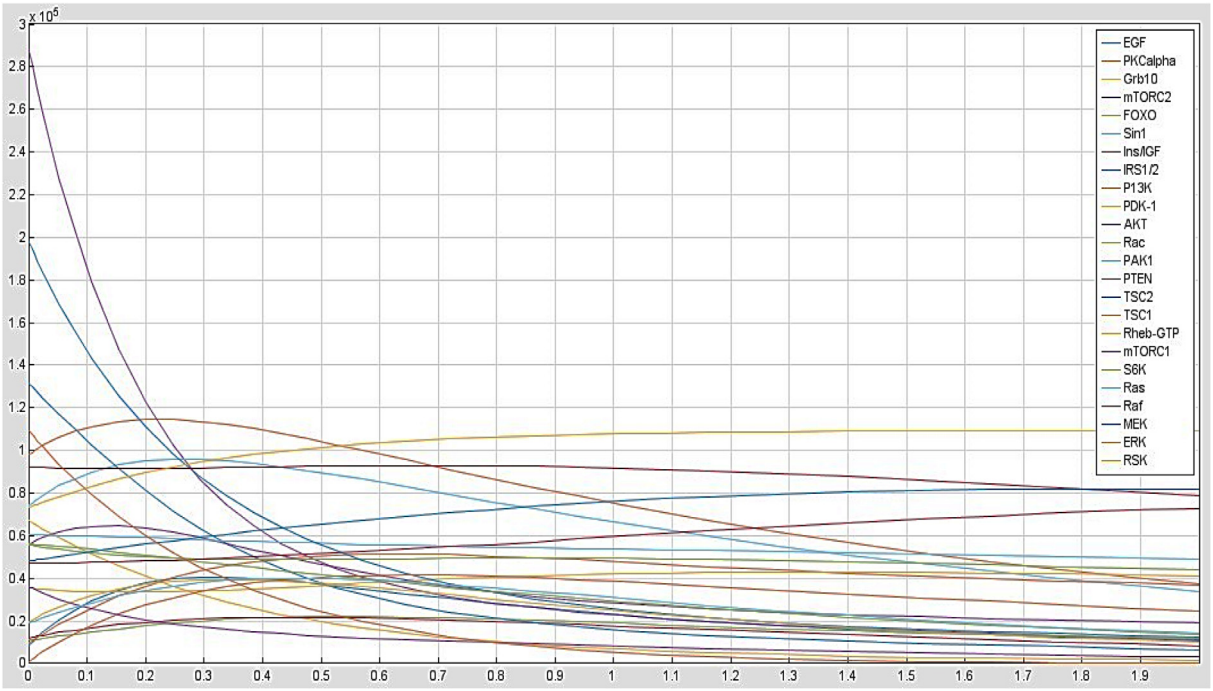

Fig. 5. Simulation graph of all the species involved in a pathway.

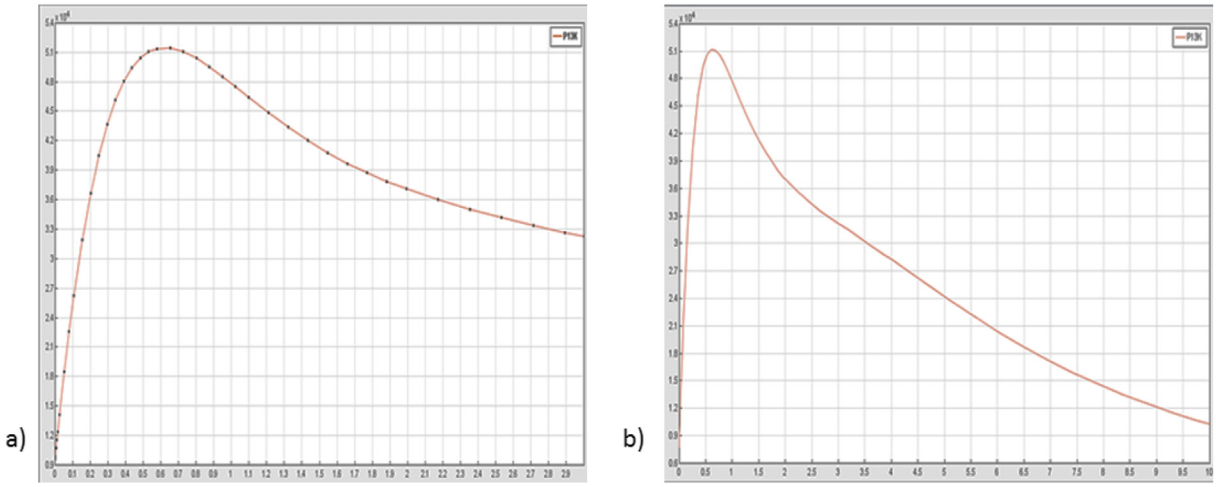

Fig. 6. (a) PI3K level without dose, (b) exponential degradation of PI3K with dose.

tion of mTORC1 reaches to zero. However, an exponential upsurge was observed in the concentration of mTORC1 after applying amygdalin dose (250 mL) as shown in Fig. 7a&b.

Active-site inhibitors enhance the activity of ERK by its over-activation and ERK concentration was found higher in cancer patients (Soares et al., 2013). Amygdalin dose shows no effect on the activity of ERK, even the dose value was increased up to 400 mL (Fig. 8a&b).

Akt is over-regulated in cancer pathways. Amygdalin dose shows neither increase nor decrease to the Akt pathway directly even the dose amount was increased up to 400 mL (Fig. 9 a&b), but it is effective indirectly by applying the dose to PI3K which is inter-connected to Akt (Mendoza et al., 2011).

Ras signaling pathways have been detected in association with a variety of cancers (Fernández-Medarde and Santos, 2011). Drug dose was applied directly on Ras node and value was set to be 300 mL, but no effect on Ras activity was observed (Fig. 10 a&b).

4. Conclusion:

Computational techniques have gained significant importance due to their proficient results. In the present study, computational technique was used to analyze the effect of amygdalin on inter connected carcinogenic pathways. The initial value of dosage (250 mL), contributed significantly in the down-regulation of cancer by effecting PI3K and mTOR pathways, which consequently effect to other carcinogenic pathways circuitously. However, amygdalin effects on ERK, Ras and AKT pathways were comparatively less, even dose value was increased. Therefore, we suggest

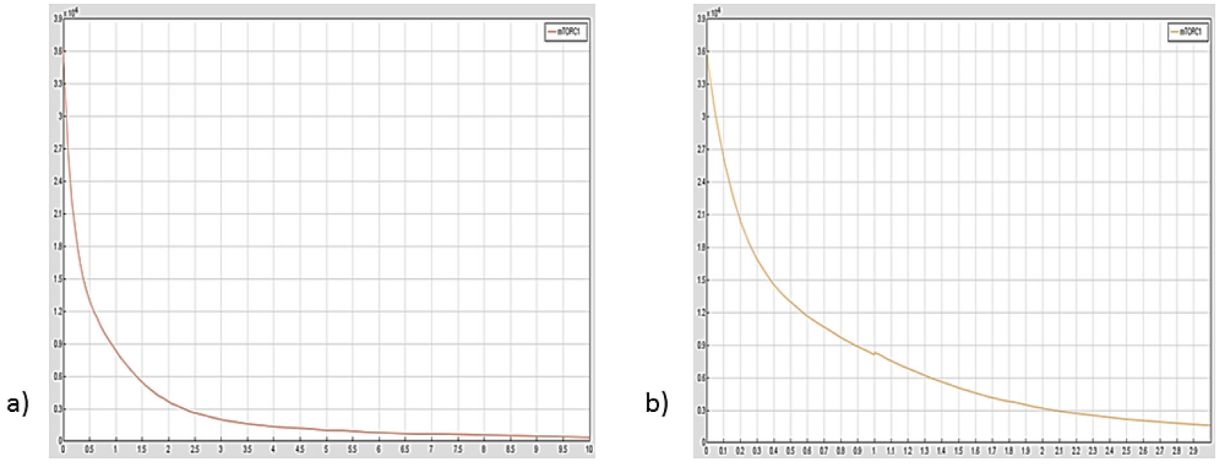

Fig. 7. (a) mTORC1 graph without dose, (b) increase of mTORC1 by applying dose.

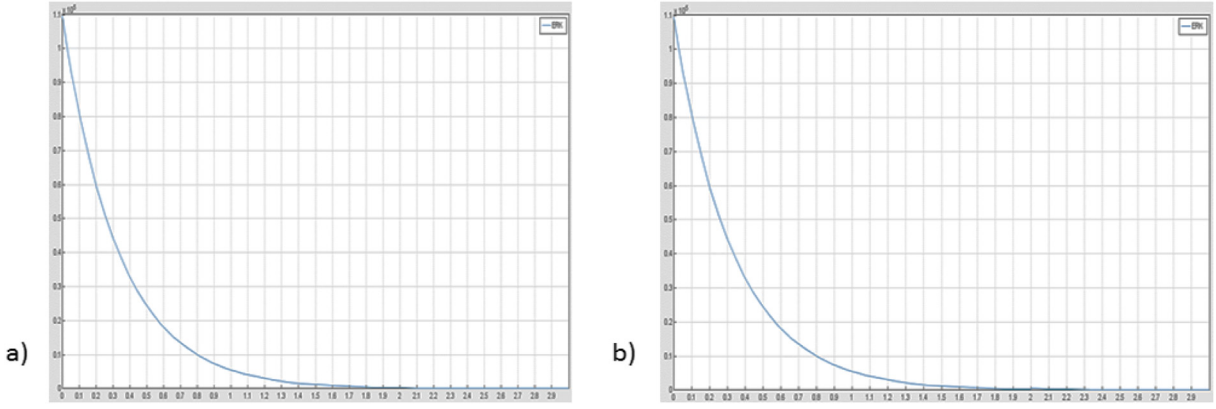

Fig. 8. (a) ERK graph without any dose (b) ERK graph with dose (250 mL).

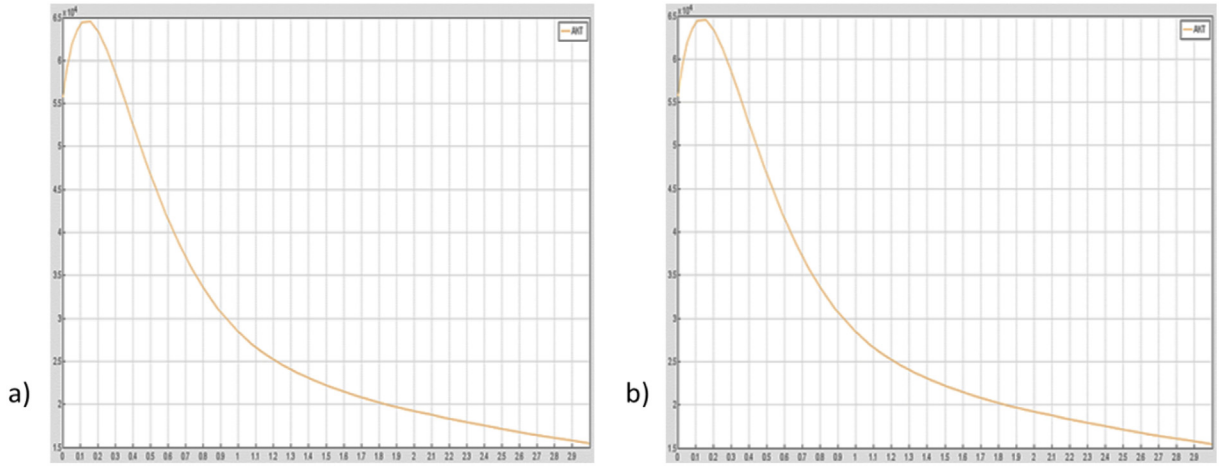

Fig. 9. (a) Akt graph without dose, (b) Akt graph with dose of 400 mL.

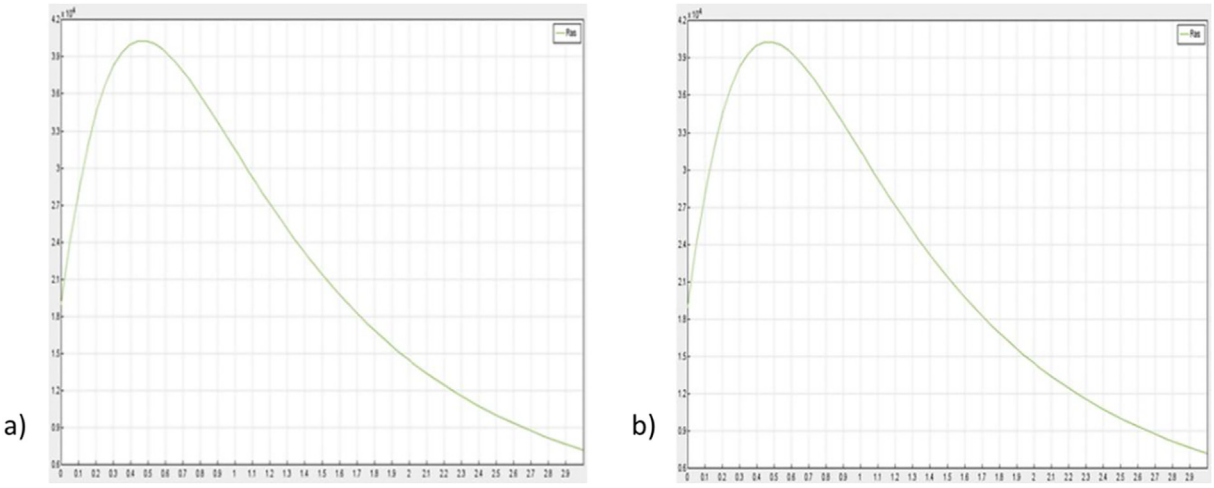

Fig. 10. Amygdalin effect on Ras pathway before (a) and after dosage (b).

that natural sources of amygdalin i.e. bitter kernels could be used in a specified amount to treat various types of cancer in human. In Addition, in vivo and *in vitro* studies could be helpful to authenticate the use of amygdalin for cancer therapy.

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Declaration of Competing Interest

None of the authors have any challenging conflict of interests.

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Further Reading

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