

**ARTICLE****An Overview on the Anticancer Potential of Punarnavine: Prediction of Drug-Like Properties****Imad Ahmad<sup>1,2</sup>, Haroon Khan<sup>2,\*</sup>, Muhammad Usman Amin<sup>1</sup>, Shah Khalid<sup>3</sup>, Tapan Behl<sup>4</sup> and Najeeb Ur Rahman<sup>1</sup>**<sup>1</sup>Department of Pharmacy, Abasyn University, Peshawar, 25000, Pakistan<sup>2</sup>Department of Pharmacy, Abdul Wali Khan University, Mardan, 23200, Pakistan<sup>3</sup>Department of Botany, Islamia College University, Peshawar, 25120, Pakistan<sup>4</sup>Chitkara College of Pharmacy, Chitkara University, Punjab, 140401, India

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**ABSTRACT**

Punarnavine (PN) has been isolated from the roots of *Boerhaavia diffusa* L. (Nyctaginaceae). It is an important medicinal plant with a vast historical ethnopharmacological background. Its identification resulted from the interrogation of 'Punarnava', a tribal medicinal product. The molecule exhibited its position as incipient anticancer therapeutic agent. The inhibition of NFκB, ATF-2, c-Fos, and CREB-1 are one of the underlying mechanisms of anticancer action along with modification of the immune system. These signalling molecules are upregulated in the cancer microenvironment. Punarnavine also modified the release of interleukins, i.e., upregulated IL-2, IL-7, IL-10, IL-12, IFN-γ, while downregulated IL-1β, IL-6, TNF-α, VEGF, and GM-CSF. Punarnavine has exhibited its anticancer potential in murine melanoma cell lines, breast cancer and its metastasis to lungs, liver, and lymph nodes by modifying apoptotic pathways and even suppressing metastatic genes like TIMP-1, TIMP-2, MMP-2, MMP-9 and VEGF. The pharmacokinetic and basic drug discovery properties are also discussed concerning *in silico* databases. This approach exhibited PN as a successful molecule for drug development. The drug-likeness, solubility, GIT absorption, drug metabolism, and bioavailability of PN are promising of its drug candidature and therefore need further detailed studies to ascertain its clinical potential. This article is a preview of the preclinical anticancer profile of Punarnavine, including the computational approach of its drug-like properties.

**KEYWORDS**

Punarnavine; plant alkaloid; therapeutic potential; drug likeness

**1 Introduction**

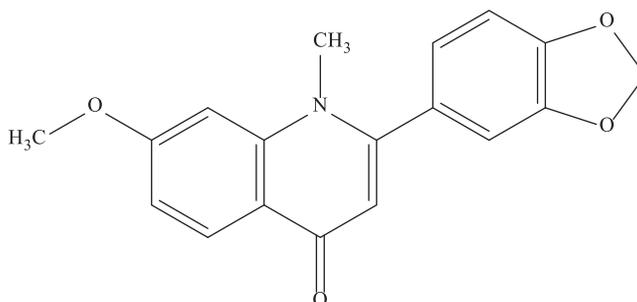
*Boerhaavia diffusa* L. (BD; family: *Nyctaginaceae*) is an important ethno-herbal medicine used in Ayurvedic and Unani system of therapeutics in many parts of the world, including India. It is widely distributed in the tropics and subtropics [1].

Medicinal properties of *Boerhaavia diffusa* have been utilized since long in the indigenous system of medicine in India. It is a perennial, creeping herb found throughout wasteland and grasslands of India; also grown in Western Africa, Angola, Ghana, Nigeria, Sudan and Australia [2]. The roots of *Boerhaavia*



*diffusa* possess diuretic, laxative, antistress, antioxidant, fibrinolytic, nephroprotective, anti-inflammatory and anticonvulsant activities [2–8]. The roots of BD is commonly known as ‘Punarnava’, which is used by numerous tribes in India for the treatment of hepatic disorders, i.e., liver enlargement, jaundice, ascites, anasarca [9,10]. Different parts of the plant are used as an appetizer, alexiteric, eye tonic, for flushing out the renal system, and to treat blood pressure [11–13].

Punarnavine (PN) is a quinolizidine alkaloid found in *B. diffusa*. It is isolated from the whole plant-in the alkaloidal fraction [10,14,15]. Syringaresinol mono- $\beta$ -D-glucoside along with PN was also isolated from roots of BD [16]. The chemical formula of PN is  $C_{18}H_{15}NO_4$  and its molecular weight is 309.32 Da (Fig. 1) [17].



**Figure 1:** Structure of Punarnavine

The purpose of this article is to summarize the preclinical anticancer potential of PN as a newly emerged drug candidate from natural source. The source of this plant is already used in traditional medicine. Therefore, this natural product will be a new approach of anticancer drug discovery.

## 2 Analysis of Punarnavine

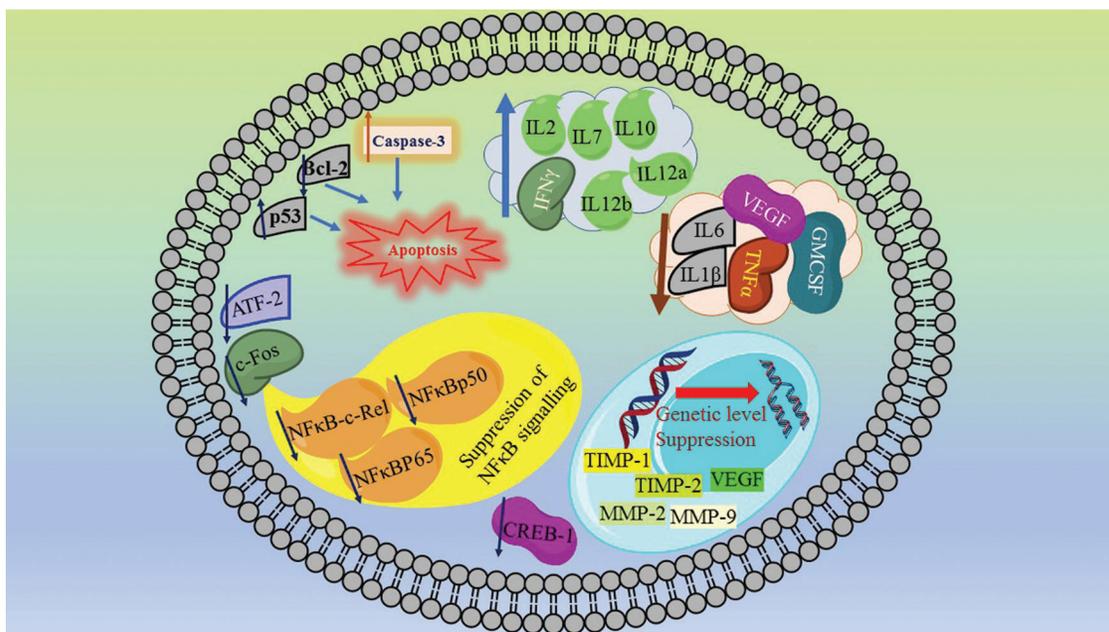
In a recent study, analytical method has been developed for PN found in plant extract [18]. A quantitative method based on high performance thin layer chromatography has been developed for the estimation of PN ( $R_f = 0.73$ ). The gradient mobile phase was used containing toluene, ethyl acetate and formic acid in the ration of 7.0:2.5:0.7 at 262 nm. This validated method was linear in a wide range [ $r^2 = 0.9971$ ] (100–1000 ng/spot), precise, and accurate with robustness. The robustness was proved with the Box–Behnken design (BBD). The limit of detection and limit of quantification was  $30.3 \text{ ng spot}^{-1}$  and  $100.0 \text{ ng spot}^{-1}$ , respectively.

## 3 Preclinical Anticancer Profile of Punarnavine

Manu et al. [18] explored the mechanism of apoptosis in B16F-10 melanoma cells. At nontoxic dose dependent concentrations PN resulted in the formation of apoptotic bodies and DNA fragmentation. This observation was confirmed by cell cycle analysis and TUNEL assays. The apoptotic genes p53 and caspase-3 were upregulated, while the antiapoptotic gene Bcl-2 was downregulated in PN treated cells. The mechanism is shown in Fig. 2. The nuclear translocation of NF $\kappa$ B-c-Rel, NF $\kappa$ Bp50, NF $\kappa$ Bp65, ATF-2, c-Fos, and CREB-1 was inhibited in PN treated B16 F-10 cells which suggest suppression of NF $\kappa$ B signalling. This is a demonstration of apoptosis induction via p53-induced caspase-3 mediated proapoptotic signalling along with suppression of NF $\kappa$ B induced Bcl-2 mediated survival signalling [19].

The NF $\kappa$ B is involved in pathological events, especially cancer. The stimulation of NF $\kappa$ B signalling pathway track towards progression and development of cancer. NF $\kappa$ B upregulation in cancer cells is also involved in resistance to chemotherapy [20–22]. Since it is known that NF $\kappa$ B stimulates expression of oncogenic miR-21 (microRNA) to inhibit PTEN (phosphatase and tensin homolog) expression, creating

CP resistance in lung cancer cells. PTEN is a cancer related protein which is encoded by PTEN gene. This PTEN significantly suppress PI3K/Akt and ends up the proliferation and growth of lung cancer cells [23]. The suppression of NF $\kappa$ B signalling is desirable for anticancer mechanism as well as to halt the inflammatory process.



**Figure 2:** Punarnavine inhibit NF $\kappa$ B signalling-suppress nuclear translocation of NF $\kappa$ B-c-Rel, NF $\kappa$ Bp50, NF $\kappa$ Bp65, ATF-2, c-Fos, and CREB-1 which ultimately ends up in the inhibition of cancerous proliferation. The activation of p53 and caspase-3, along with suppression of Bcl-2 leads to apoptosis pathway resulting in the death of cancerous cells. From genetic level the suppression of TIMP-1, TIMP-2, MMP-2, and MMP-9 and VEGF arrest the metastatic progression and proliferation of cancer cells. Further the reduction in the cytokines like VEGF, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and GM-CSF reduce tumour growth and metastasis. Increasing the level of antitumorogenic and antiangiogenic cytokines like IL-2, IL-7, IL-10, IL-12a and IL-12b further synergise the overall anticancer mechanistic spectrum of Punarnavine

Breast cancer become fatal when cancerous cells spread from site of tumour origin and colonise in distant organs. This is followed by systemic plantation wherever it is circulated *via* blood. PN has already proven its antimetastatic effect on spontaneous B16F10 pulmonary metastasis. To further extend anticancer spectrum, Kallivalappil et al. [23] evaluated its effect on one of a drastic organ-specific breast cancer. A syngenic mouse model (4T1 breast tumour model) was used for the study which mimics the staged-four human breast cancer. PN (40 mg/kg) administration to BALB/c mice reduced metastatic progression of 4T1 to the lymph nodes, liver, and lungs. This was evidenced from the histopathology of these organs. However, metastatic cell density of cultured 6-thioguanine-resistant 4T1 cells followed a declining pattern in the PN-treated group as compared to the control group. Similarly, a significant inhibition ( $p < 0.0001$ ) of the primary breast tumour growth also confirmed in the orthotopic site of induction. A concomitant increase ( $p < 0.0001$ ) in the life span of treated animals also confirmed it. Various biochemical parameters, i.e., hydroxyproline, hexosamine, uronic acid, sialic acid, and  $\gamma$ -glutamyl transferase were found reduced in the PN-treated group. The analysis of cytokines like VEGF, TNF- $\alpha$ , IL-1 $\beta$ , and GM-CSF exhibited a similar reduction pattern in PN treated group ( $p < 0.0001$ ) as compared to the control group.

Following genomics, it was also revealed that PN can suppress metastatic genes. The inhibitory effect was observed for tissue inhibitor matrix metalloproteinase-1 (TIMP-1), TIMP-2, matrix metalloproteinase-2 (MMP-2), MMP-9 and VEGF, that are involved in the metastatic process [24]. TIMPs and MMPs are involved in breast cancer prognosis [25,26]. All these findings are a summation of arresting the metastatic behaviour of breast cancer as well as its progression. The studies are carried out in a murine model, so giving strong evidence for Punarnavine as a drug candidate against breast cancer. TIMP1 is also an important promoter of human colon cancer tumorigenesis and metastasis and a potential prognostic indicator for colon cancer [27].

Immunomodulators modify the activities of immune system. Mostly, synthetic chemotherapeutic agents which are immunosuppressants produce adverse effects. The Immunomodulators derived from plant source are used generally as adaptogens and immunity enhancers with low risk of undesired effects [28,29]. In this extension, PN was also tested following this approach.

PN provoke a non-specific immune response, and increase the phagocytic activity of polymorphonuclear cells, exhibited by the engulfment of candida cells. There was enhancement in the humoral immune response in Plaque Forming Assay. PN increase the synthesis of antibodies, macrophages, and B lymphocyte subsets; bone marrow cellularity and  $\alpha$ -esterase positive cells indicating proliferation of stem cells. PN increase the mRNA gene expression of various cytokines, i.e., IL-7; IL-10; IL-12a, and IL-12b. Hence all these effects project PN among potent immunomodulatory agents. Along with these effects PN did not exhibit any toxic effect (1000 mg/kg body weight, oral dose) in mice which classify it as safe 'drug' [30].

The immune cells are capable to kill malignant cells which are abnormal and being stressed. Since they can differentiate normal cells from the cancerous cells especially from the activated and inhibited receptor expressions. Upon binding of the activated receptors, the NK cells (immunoregulatory) release pro-inflammatory cytokines, i.e., interferon (IFN)- $\gamma$ , while the cytotoxic type of NK cells directly attack the target cell [31]. Therefore, anticancer agents with precise immune molecules as targets that support oncogenesis or tumour progression are of importance. This approach is followed by various scientists as well who design anticancer immunomodulatory agents [32,33].

Manu et al. [10] studied the effect of PN on the cell-mediated immune response in metastatic condition using C57BL/6 mice model. Administration of PN increased the activity of natural killer cells, antibody-dependent cellular cytotoxicity, and antibody-dependent complement mediated cytotoxicity. Production of cytokines (IL-2, IFN- $\gamma$ ) is enhanced after PN administration as compared to the metastatic tumour-bearing control group. However, as compared to metastatic control group, the pro-inflammatory cytokines including IL-1 $\beta$ , IL-6 and TNF- $\alpha$  are lowered. This enhanced immune response provoked against B16F-10 melanoma cells' metastatic progression in mice [10]. In a similar study by Manu et al. [33] in Balb/c mice, PN (40 mg/kg i.p.) enhanced total WBC count. After PN treatment along with sheep red blood cells (antigen), the circulating antibody titre and number of plaques forming cells in the spleen were increased. The proliferation of thymocytes, splenocytes, and bone marrow cells also increased in both the presence and absence of specific mitogens. This shows the immunomodulatory activity of PN [34]. The pathways and molecular mechanisms targeted by Punarnavine in a cancerous cell are exhibited in Fig. 2.

## 4 Other Cancer Related Activities

### 4.1 Antigenotoxic Potential

The *in vivo* genotoxic potential of PN was evaluated as per OECD guideline 474, 1997. The activity was performed using the comet assay on liver, brain, lymphocytes, spleen, and bone marrow together with micronucleus test in bone marrow cells including *in vitro* chromosomal aberration test. There were no genotoxic effects observed at all doses exhibited in the comet assay, or any clastogenic effect in the

micronucleus test [35]. The three tested doses of PN inhibited DNA damage induced by cyclophosphamide for all cells evaluated. Hence, no genotoxic or clastogenic effects were identified in these experiments. However, this significant decrease in cyclophosphamide induced DNA damage is beneficial protective effect. The antigenotoxic properties of PN may be of great value in cancer prevention and pharmacological intervention [35].

#### 4.2 Antimetastatic Potential of Punarnavine

The anti-metastatic activity of PN was studied in B16F-10 melanoma cells in C57BL/6 mice. PN administration at 40 mg/kg body weight could inhibit the metastatic colony formation of melanoma in lungs; prophylactically the inhibition was 95.25%, with simultaneous administration it was 93.9% and 10 days after tumour inoculation 80.1% inhibition was observed. Survival rate of the animals bearing metastatic tumour was increased significantly in all modalities as compared to the metastatic tumour bearing untreated control group. The biochemical parameters and histopathological studies also were in line with pharmacological actions, i.e., lung collagen hydroxyl proline, hexosamine, uronic acid, serum  $\gamma$ -glutamyl transpeptidase serum sialic acid, and serum vascular endothelial growth factor (VEGF) levels. PN administration down regulated the expression of matrix metalloproteinases, i.e., MMP-2 and MMP-9. The expression of extracellular signal regulated kinases, i.e., ERK-1, ERK-2, and VEGF were also down regulated in the lung tissue of metastatic tumour bearing animals. This inhibition of MMP-2 and MMP-9 protein expression was also exhibited in gelatin zymographic analysis of B16F-10 cells [15].

#### 4.3 Anti-Angiogenic Activity

Saraswati et al. [36] determined the *in vivo* antiangiogenic activity using sponge implant angiogenesis assay and antitumor activity against Ehrlich ascites carcinoma tumour [36]. PN inhibited the migration and invasion of endothelial cell and capillary structure formation in Human umbilical vein endothelial cells (HUVECs). PN significantly inhibited the expression of MMP-2 and MMP-9 at 50  $\mu$ M in HUVECs in an *in vitro* experiment. In sponge implant assay PN also inhibited neovascularization. PN treatment exhibited a dose-dependent decrease in the ascitic fluid volume (60.94% reduction) and tumour volume (86.40% reduction) in Ehrlich ascites model at 15 mg/kg/day. There was a reduction in peritoneal angiogenesis as well. And this effect can be extended to develop a therapeutic tool for treatment of cancer [36].

All these anticancer activities and the underlying mechanisms are summarized in Tab. 1.

### 5 Prediction of Drug-Like Properties

“Drug-like” property depends upon mode of administration. The original rule of five (RO5) deal with orally active compounds and define four simple physicochemical parameter ranges, i.e.,  $MWT \leq 500$ ,  $\log P \leq 5$  H-bond donors  $\leq 5$ , and H-bond acceptors  $\leq 10$ . These properties are associated with 90% of the orally active drugs that have achieved phase II clinical status and have acceptable aqueous solubility with intestinal permeability which are the first steps in oral bioavailability [37].

The physicochemical parameters and drug like properties (Tab. 2) were calculated with SwissADME, a free web tool used in medicinal chemistry [38]. The structure of Punarnavine is composed of carbon, hydrogens, nitrogen, and oxygen. The molecular weight is 309.32 g/mol. Drugs having molecular weight  $< 500$  are more easily absorbed through git and can be formulated in oral dosage form. Rotatable bond count is a widely used ‘drug filter’ which guides that  $> 10$  rotatable bonds correlates with decreased rat oral bioavailability [39]. While PN have only 2 rotatable bonds. The mechanistic basis for the ‘rotatable bond filter’ is unclear since its count does not correlate with *in vivo* clearance rate in the rat. But still the filter is reasonable from a viewpoint of *in vitro* screening because ligand affinity decreases 0.5 kcal (average) for each two rotatable bonds [40]. The H-bond acceptor and donor are 4 and zero, respectively. Both are within accurately strict limit. Oral drugs have fewer H-bond acceptors, donors and rotatable bonds [37].

**Table 1:** Comprehensive summary of the preclinical profile of Punarnavine

Experimental model	Pharmacological effect	Mechanism of action	Effect on genes	References
Syngenic mouse 4T1 breast tumour model (stage four human breast cancer mimicry)	Anti-breast-cancer	↓ hexosamine, sialic acid, hydroxyproline, uronic acid, and $\gamma$ -glutamyl transferase. ↓ cytokines (VEGF, IL-1 $\beta$ , TNF- $\alpha$ , GM-CSF)	⊥ metastatic genes like TIMP-1, TIMP-2, MMP-2, MMP-9, and VEGF.	[24]
Albino mice	Immunomodulatory (Delayed Type Hypersensitivity)	↑ influx of mononuclear cells, ↑ phagocytic activity, bone marrow cellularity and $\alpha$ -esterase positive cells.	↑ mRNA gene expression of IL-7, IL-10, IL-12a and IL-12b.	[30,34]
Balb/c mice 40 mg/kg i.p.	Immunomodulatory	↑ total WBC count, circulating antibody titer and number of plaques forming cells in the spleen; proliferation of thymocytes, splenocytes, and bone marrow cells.		[34]
Comet assay, clastogenic effects in the micronucleus test	Antigenotoxic effect, cancer prevention	⊥ cyclophosphamide-induced DNA damage		[35]
C57BL/6 mice injected with B16F-10 melanoma cells. (40 mg/kg body weight)	Anti-pulmonary metastatic activity (both prophylactic and post-tumour inoculation)	↓ lung collagen hydroxyl proline, hexosamine, uronic acid, serum $\gamma$ -glutamyl transpeptidase, serum sialic acid, and serum VEGF levels. ⊥ MMP-2 and MMP-9 proteins' expression.	↓ MMP-2, MMP-9, ERK-1, ERK-2, and VEGF expression in the lung tissue.	[15]
Sponge implant angiogenesis assay, Ehrlich ascites carcinoma antitumour (15 mg/kg/day)	Antitumour angiogenesis	⊥ endothelial cell migration/invasion/capillary structure formation of HUVECs. ⊥ neovascularization in sponge implant assay. ↓ ascitic fluid volume (60.94%) and tumour volume (86.40%). ↓ peritoneal angiogenesis.	⊥ MMP-2 and MMP-9 expression in HUVECs <i>in vitro</i> .	[36]

(Continued)

<b>Table 1 (continued).</b>				
Experimental model	Pharmacological effect	Mechanism of action	Effect on genes	References
B16F-10 melanoma cells	Apoptosis.	Generate apoptotic bodies and DNA fragmentation. $\perp$ NF $\kappa$ B signalling (NF $\kappa$ B-c-Rel, NF $\kappa$ Bp50, NF $\kappa$ Bp65, ATF-2, c-Fos, and CREB-1).	Upregulation of p53 (apoptotic gene) and caspase 3. Downregulation of Bcl-2 (antiapoptotic gene)	[19]
C57BL/6 mice model	cell-mediated immune response against metastatic progression of B16F-10 melanoma cells.	antibody-dependent cellular cytotoxicity, Natural Killer cell activity, and antibody-dependent complement mediated cytotoxicity. $\uparrow$ production of IL-2 and IFN- $\gamma$ . $\downarrow$ pro-inflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ), $\uparrow$ TIMP-1 level.	$\uparrow$ TIMP-1 expression.	[10]

Note:  $\uparrow$ : increase,  $\downarrow$ : decrease,  $\perp$ : block or inhibit.

**Table 2:** Predicted physicochemical properties of Punarnavine

Physicochemical properties	
Formula	C <sub>18</sub> H <sub>15</sub> NO <sub>4</sub>
Molecular weight	309.32 g/mol
No. of rotatable bonds	2
No. of H-bond acceptor	4
No. of H-bond donors	0
Molar refractivity	87.46
TPSA	49.69 Å <sup>2</sup>
Lipophilicity	2.87
Log P <sub>o/w</sub> (Average of iLOGP, XLOGP <sub>3</sub> , WLOGP, MLOGP, SILICOS-IT)	
Water solubility	Moderately soluble
Drug likeness	
Lipinski rule	Yes. No violation
Medicinal chemistry	
Lead likeness	Yes
Synthetic accessibility	2.78

The molar refractivity was found to be 87.46, total polar surface area (TPSA) 49.69 Å<sup>2</sup>, Log  $P_{o/w}$  (average of iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT) 2.87, and the molecule is moderately water soluble [41,42].

The molecule satisfies all the Lipinski's drug likeness parameters and there is no violation observed. Lead likeness shows the capability to serve as 'lead' molecule in the drug discovery process. PN has not been synthesized in the lab yet. However, the swiss database predicted its synthetic accessibility of 2.78, which suggest very easy step reactions of synthesis. The difficult molecules to synthesize are those having a score of 10.

Pharmacokinetics (PK) plays an important role in achieving the pharmacological goals of drug administration. Every parameter has its own limiting criteria. Absorption is the first phase to gain access to body tissues, followed by distribution throughout body, getting biotransformation and move out *via* excretion routes. However, the PK parameters of PN are not studied. Therefore, an initial *in silico* screening is desirable (Tab. 3). The predicted pharmacokinetics with SwissADME database, revealed high GI absorption [38]. The forecast of bioavailability and permeability is very important before proceeding towards purchasing, synthesising or any advanced testing. Since a probability-based score is assigned to a drug candidate that it will have, F > 10% in rat. The Bioavailability score obtained for PN is 0.55 [43]. It was identified as permeable through blood brain barrier and not a substrate for P-glycoprotein. This implies the lower potential of developing pharmacokinetic drug interactions [44]. The molecule can inhibit some of the major Cytochrome P450 enzymes, i.e., CYP1A2, CYP2C9, CYP2D6, CYP3A4. However, it is not an inhibitor of CYP2C19. The molecule has skin permeation as well, exhibited as Log  $K_p$  -5.97 cm/s [45].

**Table 3:** Predicted Pharmacokinetics properties of Punarnavine

Pharmacokinetic parameters	
Gastrointestinal tract absorption	High
BBB permeation	Yes
P-glycoprotein substrate	No
Metabolism	
CYP1A2 inhibition	Yes
CYP2C19 inhibition	No
CYP2C9 inhibition	Yes
CYP2D6 inhibition	Yes
CYP3A4 inhibition	Yes
Log $K_p$ (Skin permeation)	-5.97 cm/s
Bioavailability score	0.55

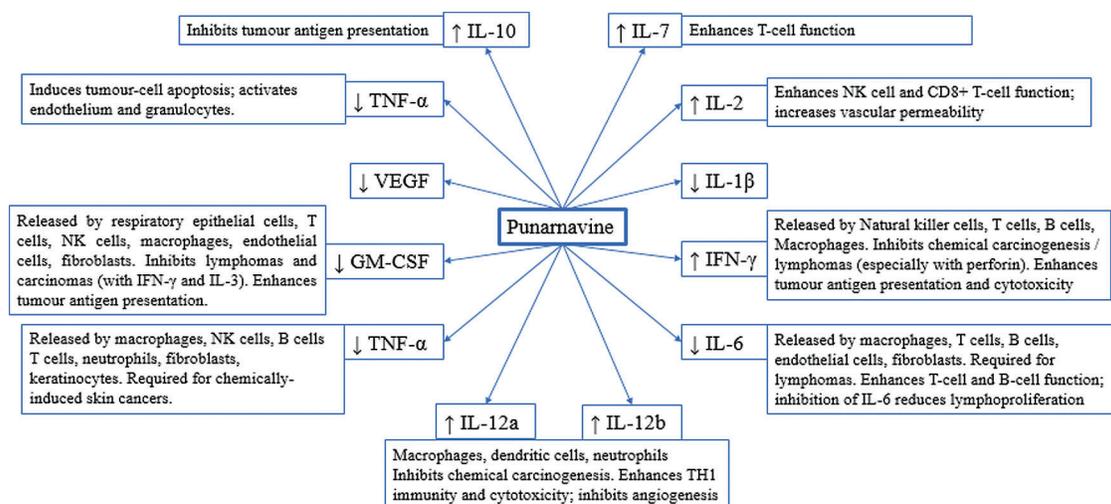
## 6 Outlook: Challenges and Approaches

In the wet lab PN has demonstrated a range of anticancer mechanisms. PN modify NFκB which is a cellular signal transduction pathway involved in physiologic and pathologic pathways. This is a family of homodimeric and/or heterodimeric transcription factors (TFs) formed by the combination of RelA, RelB, cRel, p50 and p52. The combinatorial association of RelA/p50 upregulates a broad spectrum of innate immunity genes, which encode for antimicrobial peptides (like β-defensin), cytokines (e.g., TNF) and their receptors, pro-inflammatory chemokines and cell adhesion molecules [46,47]. The inhibition of NFκBp65 nuclear translocation is also been involved in reducing apoptosis of the hippocampal neurons, alleviating their inflammation and oxidative stress [48]. Similarly, cyclic AMP-response element-binding

protein 1 (CREB-1) is a stimulus-induced 43 kDa transcription factor which is overexpressed in hematopoietic and solid tumours (CREB-associated cancers; CACs). Some of the CACs are acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic lymphatic leukaemia, Hodgkin's lymphoma, melanoma; oesophageal, gastric, hepatocellular, pancreatic, lung, breast renal cell, ovarian and prostate carcinoma, including brain tumours [49–52].

Activating Transcription Factor-2 (ATF-2) is a sequence-specific DNA binding protein. It is stress induced and upon stimulation it activates gene targets like cyclin A, cyclin D, and c-jun, which cause oncogenesis in various tissues. ATF-2 expression has been correlated with maintenance of a cancer cell phenotype [53]. Therefore, inhibition of NF $\kappa$ B, ATF-2, c-Fos, and CREB-1 by PN could be a new strategy of drug development for the treatment of human cancers and inflammatory diseases. IL-1 $\beta$ , a potent inflammatory cytokine promote tumour growth and metastasis both in animals and human breast cancer model [54]. This IL-1 $\beta$  is also one of the targets of PN, which diminish its level, promoting anticancer interplay.

PN modify the release of various cytokines which are discussed in the subsequent paragraphs and summarized in Fig. 3. PN increase the level of interleukin-2, a key cytokine approved for the treatment of cancer immunotherapy like metastatic renal cell carcinoma, and metastatic melanoma. The IL-2 might be used as monotherapy or in combination with other anticancer immunotherapies [55].



**Figure 3:** Effect of Punarnavine on cytokines, ultimately modifying the fate of cancer

Interleukin-6 is overexpressed in the tumour microenvironment and is known to be deregulated in cancer. The high level of IL-6 promotes tumorigenesis in the tumour microenvironment by modifying multiple signalling pathways, i.e., apoptosis, proliferation, invasiveness, angiogenesis, metastasis, metabolism and the survival [56]. Further, the upregulation of IL-6 promotes multidrug resistance (MDR) via activation of Janus kinases/signal transducer and activator of transcription 3 (JAK/STAT3), phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt), and mitogen-activated protein kinase pathways (Ras-MAPK) [57]. Therefore, blocking of IL-6 by PN is important from therapeutic perspective as well as to encounter chemo-resistance.

Upregulated gene IL-7 is previously correlated with human oral cancer-associated Tertiary lymphoid structure. The tertiary lymphoid structure offers a local microenvironment for cellular and humoral

immunity. The TLS support effective antigen presentation and lymphocyte activation [58,59]. Li et al. [58] favours the upregulation of IL-7 by PN, enhancing its anticancer mechanistic spectrum.

PN increase the level of Interleukin-10 (IL-10), an immune regulatory cytokine with both immunosuppressive and antiangiogenic functions. IL-10 is released by immune cells, i.e., T lymphocytes, natural killer cells, macrophages. However, it is also noted that IL-10 promotes tumour cell proliferation and metastasis via immunosuppression. IL-10 inhibits NF $\kappa$ B translocation which is in favour of its anti-inflammatory and anticancer potential [60]. These findings make IL-10 as a possible therapeutic target in the prognosis of cancer.

IL-12 is an immune-activating cytokine, which increased the infiltration by NK cells and CD8 $\alpha^+$  T cells, while decreased CD4 $^+$ Foxp3 $^+$  regulatory T cells in mice tumours [61]. IL-12 also inhibits tumour angiogenesis. IL-12 expression locally in the tumours or utilizing various dosage forms/vectors can devoid systemic toxicity. This is capable of efficient anti-tumour immunity which can be synergized with anti-angiogenic or immunomodulator drugs with safety [62].

## 7 Conclusions

Summarizing this writing by presenting Punarnavine to the drug discovery arena which has just caught in the net of a pharmaceutical scientist. The molecule has proved itself preclinically and pharmacologically, especially its anticancer drug-ability by modifying molecular mechanisms. From modifying cytokines to oncogenic pathways and oncogenes, Punarnavine can be explored further both mechanistically and pharmacologically. Its anticancer spectrum also needs to explore in several other cancers like prostate cancer, adenocarcinoma, cervical cancer etc. The pharmacokinetic profile of PN is lacking which may halt or slow down its drug development process. However, the computational approach has given a green signal for its drug candidature. Due to its small size, it can be a successful drug candidate during pharmacokinetic profiling. And if any difficulty arises, novel drug delivery approaches can be employed to solve the bioavailability problem. Chemical modification is another way of improving the existing molecule safety, efficacy, and pharmacokinetics. These are some of the challenges that need attention. Punarnavine is used as traditional medicine since long time by the name of ‘punarnava’. This article tends to rationalise its use as anticancer, based on studies related to cancer. The studies revealed about the pharmacologic effects of Punarnavine in pure form. The article is also helpful in drug development process for a medicinal chemist as well to synthesize derivatives and further rationalise the structure-activity relationship.

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