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REVIEW ARTICLE

A REVIEW ON ANTICANCER POTENTIAL OF SOME PYRANOCARBAZOLE ALKALOIDS AND ITS DERIVATIVES

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Abstract

Pyranocarbazole alkaloids isolated from natural sources various plant parts of the flower-bearing geneses plants *Murraya*, *Clausena*, *Glycosmis*, species belongs to family Rutaceae. These pyranocarbazole alkaloids showed potential anticancer activities on various cancer cell lines. In this review, we discussed the anticancer potential of pyranocarbazole alkaloids like Mahanine, Koenimbine, Koenidine, Murrayozoline, Girinimbine, Mahanimbine mainly on the basis of reported review literature. In particular, we discuss *in vitro* anticancer activities of these pyranocarbazole alkaloids and its derivatives on various cancer cell lines, plausible target, also *in vivo* activity if reported in literature discussed. These pyranocarbazole structure-based alkaloids showed very interesting anticancer profile. This review can be fruitful for further investigation of these pyranocarbazole alkaloids as anticancer drug development against various types of cancer with minimum side effects.

Introduction:-

Cancer is the second leading cause of death globally after cardiovascular disease, accounting around 10 million deaths in 2018.[1] The most common causes of cancer death in 2020 were lung (1.80 million deaths), colon and rectum (935 000 deaths), liver (830 000 deaths), stomach (769 000 deaths) and breast (685 000 deaths).[1,2,3] The cancer treatment modalities reported are surgery, radiation therapy, and systemic treatment, including chemotherapy, targeted therapy, hormonal therapy, and immunotherapy. Chemotherapy is best treatments strategy in the NCDB.[3] However, Non-selectivity and incapability to control metastasis are other major drawbacks like toxicity associated with current anticancer drugs.[4] So Development of new drug based on natural product could be the best way for treatment of cancer.

Pyranocarbazole derivatives have also been used as a potential pharmacophore for different therapeutic indications.[5] 9H-Carbazole (**1**) is an aromatic tricyclic heterocyclic molecule that is characterized by a dibenzo pyrrole ring system[5,6] (**Fig. 1**). Addition of pyran ring in fused 9H-Carbazole at first and second position give four membered pyranocarbazole[3,2-a] structure (**2**) (**Fig.1**).[7,8] Pyranocarbazole structure based alkaloids are generally isolated from the various plant parts of the flower-bearing geneses plants *Murraya*, *Clausena*, *Glycosmis*, species belongs to family Rutaceae.[8-13] Pyranocarbazole and its derivatives possess various biological activities.[8,9,10,11,12] In this review, we discussed the anticancer potential of pyranocarbazole alkaloids such as Mahanine (**3**), Koenimbine (**4**),

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Koenidine (**5**), Murrayozoline (**6**), Girinimbine (**7**), Mahanimbine (**8**) mainly and its some structure modified derivatives and checked their anticancer activities on various cancer cell lines with mechanism on the basis of report review literature (**Fig. 1**).

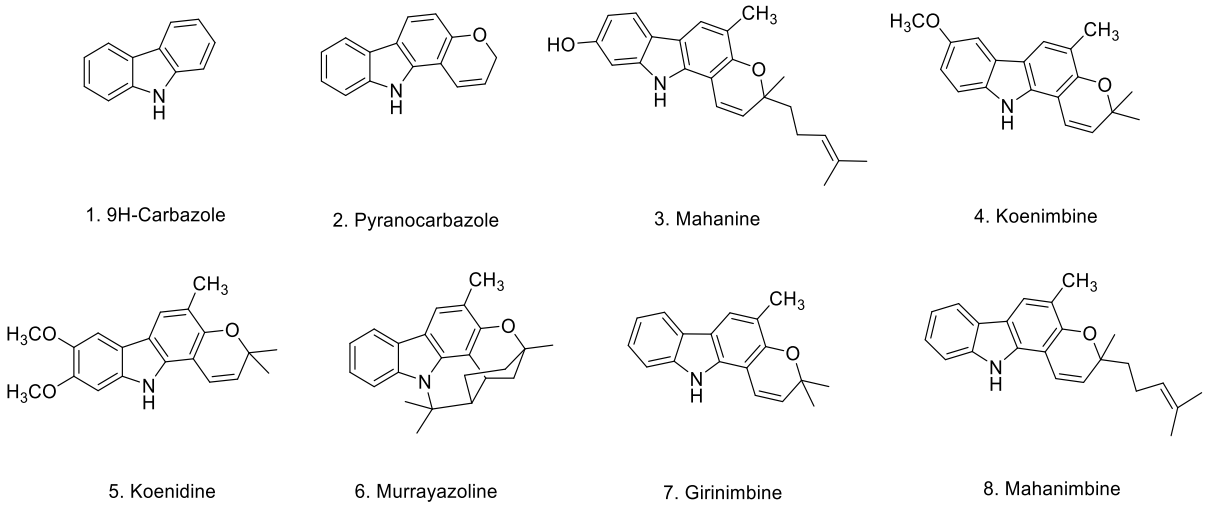


Fig 1:- Structure of 9-H-Carbazole (**1**), Pyranocarbazole (**2**) and some anticancer pyranocarbazole alkaloid (**3-8**).

Anticancer activity of plant based Pyranocarbazole structure-based derivative Mahanine

Mahanine (**3**) has a pyranocarbazole backbone (**2**) **(Fig. 1)** possessed variety of pharmacological activities like antioxidant, anti-mutagenicity, antidiabetic, antileishmanial, antimicrobial activity against gram positives, anti-inflammatory activity and also anticancer potential against various cancer cell lines.[9,14-20] IUPAC name of mahanine (**3**) is ((3,11-dihydro-3,5-dimethyl-3-(4-methyl-3-pentenyl)-pyrano[3,2-a]carbazol-9-ol)).[15,19] Mahanine alkaloid **3** presents in the edible parts of the plants *Micromelum minutum* and *Murraya koenigii*, is consumed in some parts of Southeast Asia, Thailand, and South Asia **(Fig. 1)**.[15-19]

Roy et al. and co-workers (2004) report first time the report anticancer effect of mahanine (**3**) in HL-60 human leukemic cell line.[16] Mahanine (**3**) targets at the multiple sites in the cell, increases ROS production and depolarizes mitochondrial membrane to release cytochrome c into cytosol inducing cell shrinkage, condensed and fragmented chromatin etc of cell nuclei within 4 hr, thus, increasing the accumulation of cells in deep G1 region to trigger apoptosis leading cytotoxicity (IC_{50} = 8.5 µM) in HL-60 cell line. The effect of mahanine (**3**) on the activation of caspase-1, 3-, 6, 8 and 9-like activities in HL-60 cells also investigated. Mahanine (**3**) significant increase the level of caspase-3 and -6 however, the level of caspase-8 and -9 slowly increased. Treatment of Mahanine (3) at 10 µM concentration in HL-60 cell line significantly increased ROS generation leading to apoptosis. In this study, mahanine (**3**) found a potent apoptosis inducer in HL-60 cells via caspase activation (3, 6, 8 and 9) and mitochondrial-dependent pathway.[16]

In 2004, M. K. Roy et al. group also report the anticancer potential of mahanine (**3**) on U937 glioma cell line.[21] Mahanine (**3**) leads to induce apoptosis in U937 cells which are involved in the activation of the caspases, they also include caspase-3 release of cytochrome c into cytosol, leads to loss of the mitochondrial membrane permeability as well as decreased level of cellular ATP. Cytochrome c release is important for the mitochondrial permeability. Mitochondria is the one of major target of the mahanine (**3**). As being the principle target of mahanine (**3**) leads to loss mitochondrion membrane permeability, as results it leads to caspase -3 activated and apoptosis took place. The IC_{50} value of mahanine (**3**) against U937 cell line was found to be 8.25 µM. Mahanine (**3**) altered the concentration of caspases 3 and 9 associated with mitochondria membrane potential, however, the levels of caspases 8 did not changed significantly and also significantly decreased the cellular levels of ATP in U937 cell line.[21]

In 2006, S. Sinha et al. and co-workers study the anticancer mechanism of mahanine (**3**) in two human prostate cancer cell lines viz. LNCaP (androgen-responsive) and PC3 cells (androgen-independent) respectively.[22] In cell viability assay, mahanine (**3**) showed IC_{50} value was found 2 µg/mL against both LNCaP and PC3 cell line on 72 h time period. They also first time demonstrate that mahanine (**3**) induced apoptosis in prostate cancer cell (PC3) activates caspases possibly via the deactivation of Akt and downregulation of Bcl-xL family. Therefore, cell-

survival pathway (PI3K/Akt pathway) could be a direct target of mahanine (**3**) induced apoptosis in prostate cancer cells.[22]

In 2006, **C Ito** and co-workers investigated the antiproliferative potential of some *Murraya koenigii* (MK) derived alkaloids on HL-60 cell line.[23] Only three carbazole alkaloids like mahanine (**3**), pyrayafoline-D, and murrafoline-I induced a marked decrease in cell viability with percentage 83.5%, 70.5 % and 52.0 % respectively at 30 µM concentration.[23] Interestingly, three carbazole alkaloid also induced the loss of mitochondrial membrane potential. These three alkaloids mahanine, pyrayafoline-D, and murrafoline-I also inhibited caspase-9 and caspase-3 leading to apoptosis in HL-60 cell line. This study suggests that mahanine (**3**), pyrayafoline-D, and murrafoline-I induced apoptotic effect in HL-60 cells through activation of the caspase dependent and mitochondrial dependent pathway.[23]

In 2010, **K. Bhattacharya** and co-workers investigated the apoptotic effects of mahanine (**3**) on human leukemic cells through Apo-1/Fas signalling.[24] Mahanine (**3**) increased the level of ROS production, mitochondrial membrane depolarization, a decrease in the Bcl-2/Bax ratio, the release of cytochrome c from the mitochondria to the cytosol and the subsequent activation of the caspase cascade and PARP cleavage lead to apoptosis mainly in MOLT-3 cells though mitochondrial pathways. They implanted a K562 xenograft into nude mice to establish the antitumor activity of mahanine (**3**) *in vivo*. A dose of 100 mg/kg/day of mahanine (**3**) significantly inhibited tumor growth and also reduced tumour volume after 8 days of consecutive treatment.[24]

In 2014, K. Bhattacharya et al. reported that mahanine (**3**) treated LN229 and U87MG cells (GBM cell lines) lead to G0/G1 phase cell cycle arrest in flow cytometery assay.[25] The IC_{50} values of Mahanine (3) was found in a range of 12-15 µM against LN229 and U87MG cells better as compared to curcumin 30-35 µM after 48 h using MTT assay.[25]

In 2013, Samantha et al. and co-workers synthesized modification on mahanine (**3**) and report its derivatives like methyl-mahanine (**9**), and 7-O-biotinyl-mahanine (1**0**), O-Acetyl-mahanine (**11**) (**Fig.2**).[19] In addition, the imino group at N-9 of dehydroxymahanine (**12**) was also methylated to give 9-methyldehydroxy-mahanine (1**3**). The IC_{50} values of the Mahanine (**3**) and its derivatives (**9-13**) against various cancer cell lines derived from 7 human malignant tissues was found to be in the range 7.0−18.0 µM **(Fig. 2)**.

The IC_{50} value of Mahanine (**3**) was found to be better (12-15 µM) than its various derivatives such as O-Me-mahanine, dehydroxy-mahanine and its methylated analog exhibited IC_{50} values in range 25−50 µM against various cancer cell lines glioma (T98G), pancreatic (Panc 1), lung (A549), colorectal carcinoma (HCT116), and chronic myelogenous leukemic cells (K562) respectively indicating that the changing substitution on the C-7-OH group on pyranocarbazole backbone showed decreases invitro anticancer activity.[19] DNA binding studies of mahanine (**3**) with CT- DNA was carried via various biophysical techniques like UV-vis spectroscopy, FT-IR, Calorimetry etc. and docking study showed mahanine (**3**) interacted with CT-DNA though noncovalent groove binding mode.[19,26] The computational study mahanine (**3**) with DNA showed that C-7-OH and indole NH showed established H-bond interaction with oxygen atom of the deoxyribose sugar of G9 (2.11 Å) and G5 (2.16 Å), carbonyl oxygen of base T7 (2.28 Å) respectively in the minor groove of DNA.[19] Moreover, mahanine (**3**) caused DNA damage, down regulated antiapoptotic Bcl-xL, and pro apoptotic Bid protein associated with BCl2 family and pro-caspases enzyme 7, 8, and 9 respectively in T98 G and Panc 1 cell lines.[19]

In 2018, S.S. Bhattacharya et al. group study the impact of mahanine (**3**) treated pancreatic adenocarcinoma (PDAC) cells on ER stress-mediated unfolded protein response (UPR) and Ca2+-signalling cross-talk for the survival.[27] In result, mahanine-treated MIAPaCa2 cell showed increased intracellular Ca2+ enhanced ER activity and also enhanced initiation of UPR signalling.[27]

In 2019, M. Chen et al. study antiproliferative activity of mahanine (**3**) against the glioma cells.[28] The IC_{50} value of mahanine (3) was found 7.5 µM on glioma HS 683 cells. In flow cytometry study, mahanine (**3**) treated glioma HS 683 cells arrest the G2/M phase of cell cycle. In western blotting experiment, mahanine (3) treatment to the HS 683 cells significantly decrease in the protein 205 levels of pCdc25c (Ser216), Cdc25c, pCdc2 (Tyr15), Cdc2 and cyclin B1 in HS 683 cells. Mahanine (**3**) also showed their anticancer effect through PI3K/AKT/mTOR 214 signalling pathway, it decreases the protein levels of p-PI3K, p-AKT and p-MTOR. *In vivo* potential of mahanine (**3**) also investigated in in xenografted mice model. Mahanine (**3**) treatment showed significantly decrease the level of Ki-67 and downregulated in the xenografted tumors while as the cleaved caspase-3 was increased. This study supports that mahanine can be a potential lead against gliomas.[28]

Koenimbine or Kenimbine or Koenimbin (4)

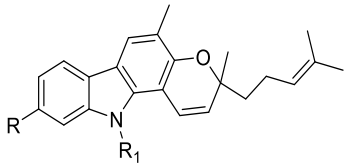
IUPAC name of Koenimbine or Koenimbin (**4**) is [3,11-Dihydro-8-methoxy-3,3,5-trimethylpyrano(3,2-a)carbazole] (**Fig. 1**).Koenimbine (**4**) is a natural product isolated from *Murraya koenigii* (L) Spreng showed potential cytotoxic effects on cancer cells.[8,29]

In 2015, F. Ahmadipour et al. and co-workers investigated anticancer potential of the Koenimbin (**4**) on MCF cell line and also target MCF7 breast cancer stem cells through apoptosis pathway *in vitro* study.[30] Treatment of MCF7 cells with Koenimbin (**4**) showed IC_{50} values 9.42±1.05 µg/mL, 7.26±0.38 µg/mL, and 4.89±0.47 µg/mL for 24, 48, and 72-hours' time period respectively. In cell cycle analysis, Koenimbin (**4**) treated MCF-7 cell line cells at 10 µg/mL concentration, it increases the percentage in the sub-G0 phase. Furtherly, Koenimbin (**4**) treated MCF7 cells induced apoptosis was mediated by cell death-transducing signals regulating the mitochondrial membrane potential by downregulating Bcl-2 and upregulating Bax, due to release of cytochrome c from the mitochondria to the cytosol. This study supports that Koenimbin (**4**) induced apoptosis effects on MCF-7 cells through multiple target pathways.[30]

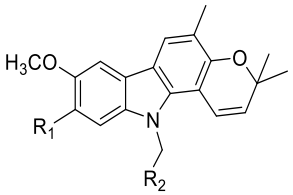
In 2016, Y. H. Hobani study the effect of koenimbine-induced DNA damage and also cell death mechanisms, using HepG2 cells.[31] The IC_{50} value of koenimbine (**4**) was found 68 ± 5.1 µM and 110 ± 7 µM on the HepG2 cells and WRL-68 cells respectively. *In vitro* cytotoxicity data, koenimbine (**4**) showed better selectivity towards HepG2 cells compared with the WRL-68 normal cell. Koenimbine (4), also significantly an increase in ROS level, decrease level of GSH, Hsp induction, DNA damage, and mitochondrial membrane potential (MMP) dysfunction was found in HepG2 cells.[31]

In 2019, O.P.S. Patel synthesized new modified pyranocarbazole derivatives based on koenimbine (**4**) and koenidine **(5)** structure (**Fig. 2)**.[8,32] All new pyranocarbazole derivative (**14aa-ao, 15aa-15ae**) were evaluated for *in vitro* anticancer potential against four human cancer cell lines viz MDA-MB-231, DU145 and PC3 cell lines. In series, one compound **14ak** showed good IC_{50} values 3.8, 7.6 and 5.8 µM, respectively on MDA-MB-231, DU145 and PC3 cell lines. In flow cytometry assay, compound **14ak** and its formulation induced G2/M arrest in MDA-MB-231 cells. Compound **14ak** and its formulation significantly decrease cellular ROS generation and also induced caspase-dependent apoptosis in MDA-MB-231 cells. Compound **14ak** also significant changing of Bax/Bcl-2 expression ratio leading to loss of mitochondrial membrane potential leading to apoptosis. Compound **14ak** also showed down-regulation of mTOR/Akt survival pathway. In SAR study, they found that the halogenated-benzyl substitution at N-9 position, C-3 Methyl and C-7 methoxy group on koenimbine (**4**) and koenidine **(5)** structure are important for potent *in vitro* anti-cancer activity. Based on koenimbine structure, compound **14ak** was found a potential candidate for anticancer activity against MDA-MB-231 cells**.**[8]

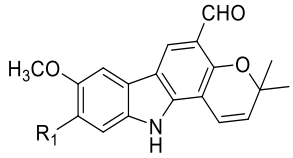
In 2020, M. Astaneh et. al group study the anticancer mechanism of koenimbine (**4**) on two colon cancer cell lines HT-29 and SW48 colon cancer cells by MTT assays.[29] *In vitro* cytotoxicity (IC_{50}) values of koenimbine were found 50 µg/ml on HT-29 and SW48 124 respectively using MTT assay. In annexin V assays, koenimbine also induction of cell apoptosis and necrosis in HT-29 and SW48 cells. Treatment of HT-19 cell line with Koenimbin (**4**) showed decrease expression of CYCLD1, TBLR1, DKK1, GSK3B and β-catenin respectively. Koenimbin (**4**) treatment in SW40 cell line significantly decrease expression of DKK1, GSK3B and β-catenin respectively.[29]



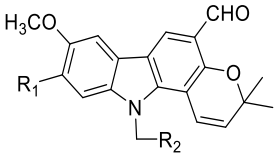
3, R = OH, R_1= H
9, R = $OCOH_3$, R $_1$= H
10,R = OCH_3, R $_1$= H
11,R = OH, R $_1$= CH_3
12, R = H, R $_1$= H
13,R = H, R $_1$= CH_3



14 aa (R_1 =H, R_2 = Phenyl), 14 ab (R_1 =H, R_2 = 3-methylphenyl)
14 ac (R_1 =H, R_2 = 2,4-diflourophenyl), 14 ad (R_1 =H, R_2 = 2,5-diflourophenyl)
14 ae (R_1 =H, R_2 = 2-bromophenyl), 14 af (R_1 =H, R_2 = acetylenyl)
14 ag (R_1 =OCH_3, R_2 = Phenyl), 14 ah (R_1 =OCH_3, R_2 = 3-methylphenyl)
14 ai (R_1 = OCH_3, R_2 = 2-bromophenyl), 14 aj (R_1 = OCH_3, R_2 = acetylenyl)
14 ak (R_1 = OCH_3, R_2 = 3-chorophenyl), 14 al (R_1 = OCH_3,, R_2 = vinyl)
14 am (R_1 = OCH_3,, R_2 = 3-methoxyphenyl), 14 an (R_1 = OCH_3, R_2 = 2,5-dichlorophenyl)
14 ao (R_1 = OCH_3, R_2 = (E)-1,2-diiodovinyl)



15 aa (R_1 =H)
15 ab (R_1 =OCH_3)



15 ac (R_1 =H, R_2 = Phenyl), 15ad (R_1 =H, R_2 = 3-chlorophenyl)
15 ae (R_1 =H, R_2 = 3-methoxyphenyl)

Fig. 2:- Structure of mahanine (**3**) and its structure modified derivative (**9-13**), structure modification in Koenimbin (**4**) and koenidine **(5)** based derivative (**14-aa-ao**) and **15-aa-15ae**

Murrayazoline (6)
The IUPAC name of murrayazoline (**6**) is 3,3,5-trimethyl-1H-pyrano[3,2-A] carbazole, **Fig. 1)**.

In 2017, A. Arun et al. and group checked the anticancer mechanism of pyranocarbazole alkaloids mainly murrayazoline (**6**) and O-methylmurrayamine were isolated from leaves of *Murraya koenigii*.[33] These two alkaloids murrayazoline (**6**) and O-methyl murrayamine showed potent *in vitro* anti-cancer activity with IC_{50} values 5.7 µM and 17.9 µM respectively on DLD-1 colon cancer cells, without showing any cytotoxicity against non-cancer HEK-293 and HaCaT cells. These pyranocarbazole alkaloids mainly murrayazoline (**6**) and O-methylmurrayamine showed alternation in cellular morphology, arrest G2/M phase of cell cycle, ROS level and loss of mitochondrial membrane depolarization of colon cancer cells. These alkaloids activated caspase-3 protein and upregulated Bax/Bcl-2 protein expression ratio leading to induction of caspase-dependent apoptosis in DLD-1 cells. Both alkaloids murrayazoline (**6**) and O-methylmurrayamine at 5.7 µM and 17.9 µM concentration showed significant decrease in Akt/mTOR phosphorylation. Akt (Protein kinase B) and MTOR (The mechanistic target of rapamycin) are also known to regulate mitochondrial physiology.[34] This study supports that murrayazoline (**6**) and O-methylmurrayamine induced mTOR/AKT downregulation and mitochondrial apoptosis. These events can be useful for the development of new drug based on pyranocarbazole alkaloids against colon cancer.[33]

Girinimbine (7)
The IPUAC name of Girinimbine (**7**) is 3,3,5-trimethyl-11H-pyrano[3,2-a]carbazole, **Fig. 1**) is a carbazole alkaloid, specifically a pyranocarbazole which was first isolated from the stem bark of *Murraya koenigii*.[35]

In 2011, S. Syam and co-workers investigate the mechanism of girinimbine (**7**) in human hepatocellular carcinoma, HepG2 cells.[36] Treatment of HepG2 cells with girinimbine (**7**) showed typical morphological features of apoptosis were observed from normal inverted microscopy and Hoechst 33342 assay. Furthermore, DNA fragmentation and elevated levels of caspase-3 with treatment of HepG2 cells with girinimbine also found. Treatment of HepG2 cells with girinimbine (**7**) also displayed a time-dependent accumulation of the Sub-G0/G1 peak (hypodiploid) and caused G0/G1-phase arrest. This study report that girinimbine (**7**) could effectively induce programmed cell death in HepG2 cells and suggests further investigations in preclinical human hepatocellular carcinoma models, especially on *in vivo* efficacy. This study can be useful to promote girinimbine (**7**) for use as an anticancer agent against hepatocellular carcinoma.[36]

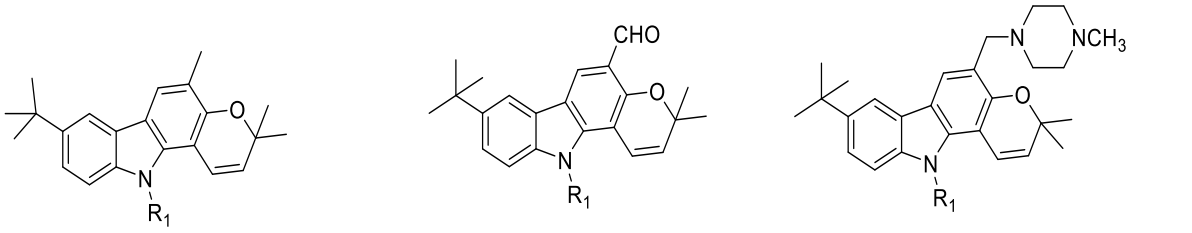
In 2013, S. Mohan and co-workers investigated the anticancer mechanism of girinimbine (**7**) on A549 lung cancer cells through apoptotic mechanistic pathway.[37] The IC_{50} value of girinimbine (**7**) was found 19.01 µM on A549 cell line using MTT assay. Treatment of girinimbine at 19.01 μM concentration in A549 cell, decrease in the nuclear area and increase in mitochondrial membrane potential and plasma membrane permeability. The release of cytochrome c leads to cell death could be affects the mitochondrial membrane potential and caspase-dependent apoptosis.[38] In this study, Girinimbine (**7**) induce apoptotic effect in the A549 cells through decrease level of Bcl-2 expression. This research study showed that girinimbine (**7**) mediates its apoptotic effect on A549 cell line and can be a useful for further drug development.[37]

In 2015, V. Iman and group report the *in vitro* and *in vivo* anti-angiogenic activity of girinimbine (7). The IC_{50} value of girinimbine was found 5±0.57 µg/mL and 20.32±0.41 µg/mL against HUVECs cells and CCD-841 cells respectively using MTT assay. Girinimbine at 20 µg/mL concentration effectively inhibited angiogenesis in zebrafish embryos following 24-hour exposure time.[35]

In 2016, V. Iman and co-workers study anticancer and anti-inflammatory activities of girinimbine (**7**).[39] Girinimbine (**7**) showed good *in vitro* cytotoxicity (IC_{50}) value 4.79±0.74 µg/mL against human colon cancer cells (HT-29) leading to apoptosis using cell viability assay without showing no cytotoxic effect on normal colon cells. In flow cytometry assay, girinimbine arrest G0/G1 phase of cell cycle in HT-29 cells. In western blot analysis, girinimbine (**7**) treated HT-29 cells induce apoptosis by changing the regulation of apoptotic proteins like Bax and BCl2, along with upregulation of caspases 9 and 3/7 and loss of mitochondrial membrane potential. Oral administration of girinimbine reduced both leukocyte migration (mainly of neutrophils), and pro-inflammatory cytokine levels (interleukin-1beta and tumor necrosis factor-alpha) in mice with carrageenan-induced peritonitis model. These findings strongly suggest that girinimbine (**7**) could act as a chemo preventive and/or chemotherapeutic agent by inducing apoptosis while also reduce inflammation.[39]

In 2020, Qiu Xin et al. investigated the effects of girinimbine (7) on cell proliferation, cell migration, and apoptosis in human ovarian cancer cells (SKOV3) *in vitro* via the Phosphatidylinositol-3-Kinase (PI3K)/Akt and the Mammalian Target of Rapamycin (mTOR) and Wnt/β-Catenin Signaling Pathways.[40] The IC_{50} value of girinimbine (7) was found 15 µM on SKOV3 ovarian cancer cell line.[40]

In 2021, Ke Liu and co. workers design, synthesized a series of pyrano[3,2-a]carbazole alkaloids based on girinimbine (7) structure modification (**Fig. 3**).[9] Anticancer activity of all compounds (**16, 16a-j, 17, 17, 17a, 17 c, 17f, 17 i, and 18, 18, 18a, 18 c, 18 f, 18 i**) were evaluated against the ten cancer cell lines viz. skin squamous cell carcinoma (A431), ovarian cancer (A2780), cervical cancer (HeLa), renal cancer (Ketr3), pancreatic cancer (SW1990), gastric cancer (BGC823), lung cancer (H460), liver cancer (H7402), colon cancer (HCT-8) and breast cancer (MCF-7) using the MTT assay. Among them, compounds **18** and **18i** with N-methyl piperazine showed potential anticancer activity against MCF-7 cell lines with the IC_{50} values of 1.77 and 4.32 µM respectively compare to girinimbine (IC_{50} = 49.76 µM) (**Fig. 3**). In flow cytometry assay, treatment of compounds 18 and 18i with MCF-7 cell line arrested G2/M and S phases of cell cycle. This study supports that pyrano[3,2-a]carbazole alkaloids modified derivatives based on girinimbine (**7)** structure with N-methyl piperazine showed potential anticancer activity. *In vitro* anti-proliferative activity data showed that existence of N-methylpiperazinyl group at C-3 position were necessary for potent anticancer activity. This study showed useful points to optimization of these girinimbine (7) structure based modified pyranocarbazole alkaloids (**16, 16a-j, 17, 17, 17a, 17 c, 17f, 17 i, and 18, 18, 18a, 18 c, 18 f, 18 i**) found potential anticancer activities for further drug development.[9]



16 (R_1= H)
16 a (R_1= benzyl)
16 b (R_1= 2-methylbenzyl)
16 c (R_1= 3-methylbenzyl)
16 d (R_1= 4-methylbenzyl)
16 e (R_1= 2-chlorobenzyl)
16 f (R_1= 3-chlorobenzyl)
16 g (R_1= 4-chlorobenzyl)
16 h (R_1= 2-flourobenzyl)
16 i (R_1= 3-flourobenzyl)
16 j (R_1= 4-methoxybenzyl)

17 (R_1 = H)
17 a (R_1= benzyl)
17 c(R_1 = 3-methylbenzyl)
17 f (R_1 = 3-chlorobenzyl)
17 i (R_1 = 3-flourobenzyl)

18 (R_1 = H)
18 a (R_1= benzyl)
18 c(R_1 = 3-methylbenzyl)
18 f (R_1 = 3-chlorobenzyl)
18 i (R_1 = 3-flourobenzyl)

Fig.3:- Structure modification in girinimbine *(7)* based derivative (**16, 16a-j, 17, 17 a, c, f, i, 18, 18 a, c, f, i)**

Mahanimbine (8)

The IPUAC name of Mahanimibine (8) is 3,3,5-trimethyl-11H-pyrano[3,2-a]carbazole, **Fig. 1**) is a carbazole alkaloid, specifically a pyranocarbazole which was first isolated from the stem bark of *Murraya koenigii*.[41]

In 2018, C. Pie at al. report Mahanimbine (**8**) inhibited the cell proliferation in various types of pancreatic cancer cells also showed lower cytotoxicity on normal cells.[42] The IC_{50} of Mahanimbine (**8**) was found in a range 3.5 to 64 μM against the pancreatic cancer cell lines by MTT assay. The lowest IC_{50} was found 3.5 μM against Capan-2 and SW119 pancreatic cancer cell lines. Treatment of Capan-2 and SW119 cells respectively with Mahanimbine (**7**) arrest G_0/G_1 cell cycle phase in flow cytometry assay. Mahanimbine (**8**) showed apoptotic effect through decrease level of Bcl-2 and enhancement of the Bax expression. Further, Mahanimbine (**8**) also inhibited the AKT/mTOR and STAT3 inhibition in the Capan-2 and SW119 pancreatic cancer cells. The effects of the Mahanimbine (**8**) were also examined for the migration activity on the Capan-2 and SW119 pancreatic cancer cells. In this study, Mahanimbine (**8**) also inhibit the motility and migration against pancreatic cancer cell lines. This study supports that mahanimbine (**8**) could be potential candidate against Capan-2 and SW119 pancreatic cancer cell lines.[42]

In 2020, Xie, H. et al. and co-workers investigated the anticancer potential of mahanimbine (**8**) on human bladder cancer cells.[43] The IC_{50} value of mahanimbine (**8**) was found 32.5 μM on human bladder cell line. Mahanimbine (**8**) mediated apoptotic cell death in human bladder cancer cell leads to increase in Bax and decrease Bcl-2 levels. Treatment of human bladder cancer cell with *mahanimbine (8)* arrest the G0/G1 cell cycle arrest in flow cytometry analysis in dose-dependent manner. Mahanimbine (**8**) also triggered autophagy due to increase in the level of LC3II expression and decrease the level of p62 expression. This research study supports mahanimbine (**8**) natural product can be a promising anticancer potential against human bladder cancer cells.[43]

Conclusion:-

In last twenty years, various researcher groups report anticancer activities of pyranocarbazole based alkaloids. In this review we discussed mainly pyranocarbazole alkaloids like mahanine, girinimbine, Koenimbine, Koenidine, murrayozoline, mahanimbine showed potential anticancer activities based on review literature. These pyranocarbazole alkaloids showed interesting anticancer profile on various cancer cell lines. We discuss thier *in vitro* anticancer profile, structure modification, anticancer mechanism and *in vivo* activity of some pyranocarbazole alkaloids based on reported review literature. These pyranocarbazole alkaloids showed potential anticancer activities leads to apoptosis through multiple targets like apoptosis, cell cycle arrest, DNA fragmentation, caspase enzyme 3,6, 8 and 9 inhibition, ROS production, DNA damage, STAT 3 inhibition, and alteration of Bax/Bcl-2 expression ratio leads to change in mitochondrial membrane potential (MMP). The pharmacological potential of the pyranocarbazole

alkaloids showed great interest to medicinal and synthetic chemist for design and development of new drugs based on natural products with minimum side effects.[44,45] This review will encourage to development of these pyranocarbazole alkaloids against various types of cancer due to potential anticancer activities with minimum toxicity.

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Conflict of interest

The authors have no conflict of interest for this paper.

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