A Review on Biological Properties of Nuphar Alkaloids

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Abstract
One of the recently identified alkaloids for the cancer treatment is Nuphar alkaloids. For past few years, interest has grown with in Nuphar alkaloids that have been extracted from diverse Nuphar species. The common structure in all Nuphar alkaloids is the presence of a methyl group embedded at C-3 position of trisubstituted piperidine ring and a furyl substituent at C-6 position. The Nuphar alkaloid has outstanding bioactivity, including immunosuppression and anti-metastatic properties, thanks to the electrophilic sulphur atom in the thiaspirane pharmacophore. The absence of the 6-hydroxy substituent in substances like nupharidine, deoxynupharidine, and neothiobinupharidine results in minimal cytotoxicity and no discernible influence on the production of plaque-forming cells. Hence, this literature review summarizes several Nuphar alkaloids isolated, and their biological characteristics identified so far.

Keywords: 6-hydroxy group; Cytotoxicity; Plaque-forming cell formation; Sulphur atom; Yellow pond lily

INTRODUCTION

Nuphar alkaloids are one of the emerging alkaloids in the treatment of cancer (Wiart C, 2013). In 1879, Nuphar alkaloids were identified by Dragendorff for the first time in the rhizomes of *Nuphar Lutea*. Since then, many Nuphar species, including *N. Lutea, N. Pumila, N. Japonica,* and *N. Speciosum*, have yielded alkaloids such nupharine, nupharidine, deoxynupharidine, nympheine, and others (Wrobel JT, 1967). Sesquiterpenoid and triterpenoid alkaloids with indolizidine, piperidine, and quinolizidine ring structures make up Nuphar plants (Davis FA et al., 2006; Raghavan S et al., 2016). All Nuphar alkaloids share the same structural characteristics, which are defined as

presence of methyl moiety at C-3 position of the trisubstituted piperidine ring and 3-furyl substituent at C-6 position (Goodenough KM et al., 2005). Additionally, they include an electrophilic sulphur atom in the thiaspirane pharmacophore, which significantly increases the Nuphar alkaloid's bioactivity (Marcaurelle LA and Mulvihill MJ, 2016). This review summarizes various Nuphar alkaloids isolated, and their biological properties identified so far.

METHODS

A thorough assessment of the literature has been conducted using Google and search engines like PubMed (1962 to 2022). Downloading and retrieving published information about Nuphar alkaloids and their biological characteristics, such as immunosuppression, antioxidant activity, cytotoxicity, etc., was the major goal.

DISCUSSION

1. Nupharine

Nupharine is an amorphous Nuphar alkaloid with the chemical formula of $C_{18}H_{24}O_2N_2$ and a melting point of 65^{o}C (Figure 1; Wrobel JT, 1967). It induces a trance-like state, has opium-like effects and acts as antispasmodic thereby aiding appetite by suppressing nausea (Doctorlib, 2022; Johnson LM, 2006). Nupharine, found in the flowers of *Nymphaea alba* when administered to frogs, mice, rats, guinea pigs, and pigeons is reported to produce paralysis of the cerebrum and respiratory poisoning (Oliver-Bever B, 1986).

1.1 Antioxidant property

The blossoms of *N. alba*, commonly known as the European White Waterlily or White Lotus, contain the active alkaloids nupharine and nymphaeine, which, according to some accounts, are sedatives and which do/do not increase sexual desires. When compared with aqueous floral extract, ethanolic floral extract at a concentration of 125 g/ml demonstrated the greatest suppression of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity. Furthermore, at concentrations of 125 g/ml and 400 g/ml, ethanolic floral extract demonstrated considerable nitric oxide scavenging activity and hydroxyl radical scavenging activity, respectively. In conclusion, as compared to aqueous floral extract, the ethanolic flower extract demonstrated significant antioxidant activity. This study's drawback is that it does not precisely state which floral component—nupharine, nymphaeine, or a combination, is truly accountable for the antioxidant action (Madhusudhanan N et al., 2011).

2. Nupharidine and Deoxynupharidine

Soon after nupharine isolation, the rhizome of *N. Japonicum* was investigated. In China and Japan, the rhizomes of *N. Japonicum* are employed as diuretics and stomach analgesics in traditional medicine (Goodenough KM et al., 2005). They are also employed as a form of crude medication for the treatment of edema, irregular menstruation, and blood purification and sedation (Okamura S et al., 2015). Nupharidine, with the chemical formula $C_{15}H_{23}O_2N$ and a melting point of

222^{o}C, was isolated from *N. Japonicum* by Arima and Takahashi (Figure 1). Deoxynupharidine, an immunosuppressant, was discovered in *N. Japonicum* in 1943 by Kotake et al. Its melting point is 21–22^{o}C and its chemical formula is $C^{15}H^{23}ON$ (Figure 1; Wrobel JT, 1967). When obtained from *N. pumila* rhizomes, nupharidine and deoxynupharidine were reported to be inactive (Matsuda H et al., 2001).

2.1 Immunosuppressive property

A study on immunosuppressive activity of methanolic extract of *N. Pumila* was conducted in 2001 using plaque forming cell (PFC) assay. Nupharidine and deoxynupharidine, monomeric sesquiterpene alkaloids without hydroxyl groups, at a concentration of 1 µM, exhibited no discernible impact on the production of anti-sheep erythrocyte PFC in mouse splenocytes (Matsuda H et al., 2001).

2.2 Anti-metastatic property

Cancer metastasis is the primary cause of mortality in cancer patients and its blockade has been considered to enhance survival of cancer patients. In this regard, an *in-vitro* test of methanolic rhizome extract of *N. Pumila* has been tested on the B16 melanoma cells spread on collagen-coated filters. According to this study, monomeric sesquiterpene alkaloids like nupharidine and deoxynupharidine poorly hindered the invasion of B16 melanoma cells embedded on collagen-coated filters at a concentration of 100 µM. (The percentage inhibition for nupharidine was 47.2% and for deoxynupharidine was 19.1%) (Matsuda H et al., 2003). [13]

2.3 Cytotoxic property

A study by Matsuda *et al*. in 2006 reported the cytotoxic effects of the alkaloid fraction and methanolic extract from the rhizomes of *N. Pumila* on human leukaemia cell (U937), mouse melanoma cell (B16F10), and human fibroblast (HT1080). According to this study nupharidine at 10 µM concentration, when incubated with U937, HT1080 and B16F10 for 72 hours, has shown weak cytotoxicity (the percentage inhibition for nupharidine was 11.5%, 4.1% and −9%, respectively). This investigation also revealed that 7-epideoxynupharidine at 10 µM concentration, when incubated with HT1080, B16F10 and U937 for 72 hours, has shown weak cytotoxicity (the percentage inhibition for 7-epideoxynupharidine was 8%, −7.9% and −10.1%, respectively) (Matsuda H et al., 2006).

3. 6-Hydroxythiobinupharidine and 6,6'-Dihydroxythiobinupharidine

The chemical formula of 6-hydroxythiobinupharidine is $C_{30}H_{42}N_2O_3S$ and for 6,6'-dihydroxythiobinupharidine is $C_{30}H_{42}N_2O_4S$ (Figure 1; Wrobel JT, 1967). Both of the constituents are present in *N. Pumila* and *N. Japonica*.

3.1 Anti-metastatic property

The most aggressive kind of bone malignancy, osteosarcoma, is challenging to treat in individuals who have lung metastases. In this regard, 6-hydroxythiobinupharidine was investigated for the inhibitory action on metastases of

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LM8 osteosarcoma cells. The study reported that LM8 cells were significantly inhibited ($p<0.001$) at a dose of 3 µM or higher, but not at a dose of 1 µM or lower. Furthermore, the number of migrated LM8 cells were significantly lower ($p<0.01$) at concentrations of 1 µM. According to western blotting results, phosphorylated Cofilin and LIM Domain Kinase 1 expression are downregulated, which prevents the metastases of LM8 osteosarcoma cells. Thus, it may be said that 6-hydroxythiobinupharidine is a potent inhibitor of metastases and a cancer preventative (Yoshizawa M et al., 2019).

The anti-metastatic activity of 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine obtained from methanolic extract of *N. Pumilum* was evaluated and it was observed that both 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine exhibited strong activity against colonization of B16 melanoma cells *in-vitro* with IC_{50} value for 6-hydroxythiobinupharidine being 0.029 µM and 0.087 µM for 6,6'-dihydroxythiobinupharidine. Cytotoxic effect was observed in both compounds at 10 µM and 100 µM concentration. 6-hydroxythiobinupharidine (5 mg/kg/d, po) effectively reduced lung tumour development in mice by more than 90% ten days after infusion with B16 melanoma cells. As a result, it was determined that the thiohemiaminal structure with the 6-hydroxyl group appeared to be necessary for significant action, and this finding may help researchers design anti-metastatic medications (Matsuda H et al., 2003).

3.2 Anti-apoptotic property

In the past, Nuphar alkaloids derived from the *N. Pumila, N. Japonicum,* and *N. Lutea* have triggered apoptosis in mammalian cells in less than an hour, making them potentially the quickest inducers. Apoptosis plays a critical role in the treatment of cancer. In this context, 6-hydroxythiobinupharidine was investigated for the apoptotic action on various cell lines. 6-hydroxythiobinupharidine, irrespective of the cell type, induced rapid apoptosis within 1 hour at a concentration of 10 µM in the U937 human leukemia cells, lymphocytic leukemia cells and carcinomas. However, 6-hydroxythiobinupharidine was unable to cause platelets to undergo apoptosis. The study came to the conclusion that 6-hydroxythiobinupharidine promoted fast apoptosis in several cell lines without the approach of BAX/BAK pathway while preserving the potential of mitochondrial membrane (Mallick DJ et al., 2019). The nuclear factor κB (NFκB) family of transcription factors plays a pivotal role in apoptosis of cancer cells. A study using methanolic extract of *N. Lutea* for inhibition of NF-κB induction was conducted and reported that 6-hydroxythiobinupharidine and 6-hydroxythionuphlutine significantly inhibited NF-κB and these compounds along with cisplatin and etoposide demonstrated as potential sensitizers in chemotherapy (Lacharity JJ et al., 2017; Ozer J et al., 2009; Ozer J et al., 2017).

The study by Matsuda *et al.* in 2006 also reported the cytotoxic effect of 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine using U937, B16F10, and HT1080 cells. At 10 µM concentration, when both compounds were incubated with these cell lines for 72 hours, they exhibited substantial cytotoxicity (94.3%, 98.7%, 99.2% and 81.6%, 54.8%, 75.3%, respectively). When 6-hydroxythiobinupharidine's ability to cause apoptosis in U937 cells was investigated, it was found that apoptotic bodies were generated within an hour after dosing. It could be a novel candidate for apoptosis-inducing chemopreventive medicines as no molecule with immediate apoptosis-inducing action within 1 h has yet to be discovered (Matsuda H et al., 2006).

3.3 Cross-resistant property

Multi-drug resistance is the major problem with anti-cancer drugs and secondary metabolites from plants are emerging as prospective medicines for cancer therapy. CCRF-CEM cells and CEM/ADR5000 cells were utilised to investigate the cytotoxicity of *N. Pumila* and *N. Japonicum* methanolic extract. It was observed that 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine were more efficient against CCRF-CEM cells than CEM/ADR5000 cells. In comparison to doxorubicin, 6,6'-dihydroxythiobinupharidine had less cytotoxic effects on CCRF-CEM cells. These results suggest that 6,6'-dihydroxythiobinupharidine was cross-resistant to multidrug-resistant CEM/ADR5000 cells (Fukaya M, 2018).

3.4 Antileukemic property

N. lutea extract has been shown to have antileukemic properties in human acute myeloid leukaemia (AML) cell lines by a recent *in-vitro* investigation. *N. Lutea* extract when incubated with U937, HL60, and KG-1a human AML cells for 72 hours have shown strong inhibition for the growth (IC_{50} values are 0.90±0.58, 1.09±0.13 and 0.89±0.05 µg/mL, respectively). At a concentration of 2.5 µg/mL, pure 6,6'-dihydroxythiobinupharidine considerably outperformed N. lutea extract in lowering the number of viable cells and poly ADP ribose polymerase cleavage in U937 cells and HL60 cells after 4 hours of incubation. Further investigation between pure 6,6'-dihydroxythiobinupharidine and *N. Lutea* extract lead to the conclusion that 6,6'-dihydroxythiobinupharidine was more efficacious when compared with *N. Lutea* extract and produced reactive oxygen species (ROS) (Muduli S, et al., 2022).

3.5 Anti-leishmanial property

Leishmaniasis remains problematic in developing countries and the number of effective drugs available is still very limited. On this note, *N. Lutea* extract containing 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine was investigated for anti-leishmanial activity and was observed that Inducible nitric oxide synthase generation and transcription factor NF- κB activation were both involved in the anti-leishmanial action. Furthermore,

it was also observed that *N. Lutea* extract almost completely inhibited the macrophage respiratory burst activity because of its anti-oxidant property. The limitation of the study was that it does not specify which constituent is responsible for the anti-leishmanial activity and anti-oxidant property (Ozer L et al., 2010).

3.6 Immunosuppressive activity

The immunosuppressive activity of 6-hydroxythiobinupharidine and 6,6'-dihydroxythiobinupharidine using PFC assay was studied in 2001. When these compounds were incubated with splenocyte culture with sheep red blood cells (SRBC) for 4 days, they significantly prevented the plaque occurrence at a concentration of 1 µM. These compounds were also examined for secondary PFC formation and appeared to directly inhibit anti-SRBC antibodies in vaccinated B cells at a concentration of 1 µM (Matsuda H et al., 2001).

3.7 Anti-fungal property

A study was conducted to observe the anti-fungal properties of 6,6'-dihydroxythiobinupharidine *in-vitro* against eight human pathogenic fungi. 6,6'-dihydroxythiobinupharidine inhibited the growth of *H. capsulatum, M. gypseum* and *M. canis* at a concentration of 100 µg/ml for 3 weeks as well that of *Trichophyton tonsurans* and *T. mentagrophytes* for 5 weeks; however, at the same concentration, it completely suppressed the growth of *Blastomyces dermatitidis*. Some strains of *B. dermatitidis* and *Histoplasma capsulatum* may be resistant to at mycelial growth when 6,6'-dihydroxythiobinupharidine is used at a concentration of 40 µg/ml. Thus, it was determined that in-vitro antifungal capabilities of 6,6'-dihydroxythiobinupharidine exist. 6,6'-dihydroxythiobinupharidine did not cause any fatalities in mice when given intraperitoneally at doses of 100 and 200 mg/kg for 30 days, but at 400 mg/kg, four out of six mice perished within that time frame (Cullen WP et al., 1973).

3.8 Anti-bacterial property

Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) are two examples of multidrug-resistant bacteria that can cause significant infections. 6,6'-dihydroxythiobinupharidine is emerging as a strong contender for new antibacterial medicines. The minimum inhibitory concentration of 6,6'-dihydroxythiobinupharidine against diverse MRSA and VRE bacteria is 1-4 µg/ml. Additionally, 6,6'-dihydroxythiobinupharidine was also found to suppress deoxyribonucleic acid (DNA) topoisomerase IV in *S. aureus* (IC_{50} was 10–15 µM), but DNA gyrase was not suppressed. However, there was only very minimal cross-resistance against norfloxacin-resistant S. aureus, suggesting that 6,6'-dihydroxythiobinupharidine may inhibit sites except topoisomerase IV (Okamura S et al., 2015).

3.9 Anti-microorganism property

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Aggregatibacter actinomycetemcomitans, JP2 clone (Aa-JP2)-caused periodontitis is difficult to treat since non-surgical methods of eradicating this pathogen are ineffective. To overcome this problem, 6,6'-dihydroxythiobinupharidine was investigated for *in-vitro* antimicrobial capability against Aa-JP2 and its impact on neutrophils. According to the study, 6,6'-dihydroxythiobinupharidine did not directly produce bactericidal effect on JP2 however, it did speed up neutrophil clearance of Aa-JP2. It elevated the levels of neutrophils priming markers, specifically intracellular IL-1β, DECTIN- 2 and iCAM1. Additionally it also increased ROS production, neutrophil phagocytosis and extracellular traps formation during JP2 infection. As a result, it was determined that 6,6'-dihydroxythiobinupharidine might be employed as an adjuvant in the management of Aa- JP2 periodontitis, however this needs to be initially verified by pre-clinical and clinical trials (Levy DH et al., 2019).

3.10 Anti-topoisomerase property

Type II topoisomerase removes knots and tangles from the genetic material and modulate torsional stress in DNA. Isoforms of type II topoisomerase such as topoisomerase IIα and IIβ are the focus for a few of anti-cancer medications as they are essential for cell survival, termination of DNA replication etc. 6,6'-dihydroxythiobinupharidine used in this regard enhanced DNA cleavage largely mostly by topoisomerase IIα (~8-fold) when compared with topoisomerase IIβ (~3-fold). More topoisomerase IIα studies show that the 6,6'-dihydroxythiobinupharidine functions by metastasizing the enzyme outside of the DNA cleavage-ligation active region and this activity is aided by N-terminal domain of the protein (Dalvie ED et al., 2019). [25]

3.11 Anti-PKC activity

Protein kinase C (PKC) family control diverse cellular processes, such as apoptosis, transcription, translation etc and are linked to numerous diseases, including cancer. 6,6'-dihydroxythiobinupharidine is reported to be an inhibitor of PKC isoforms. PKCs were inhibited by 6,6'-dihydroxythiobinupharidine in a dose-dependent manner, having the most profound effect on conventional PKCα (IC_{50} = 0.174 µM) and PKCγ (IC_{50} = 0.168 µM). Other PKC isoforms such as PKCε, PKCη, PKCζ and PKCδ had an IC_{50} of 14.23 µM, >19 µM, 18.6 µM and >19 µM. According to *in-vitro* kinase activity data, molecular docking study showed that 6,6'-dihydroxythiobinupharidine had the greatest affinity for traditional PKCs. It was determined as a result that 6,6'-dihydroxythiobinupharidine alters important cellular signal transduction pathways important to disease biology, including cancer (Waidha K et al., 2021a).

3.12 Antiproteases property

Proteases are extensively dispersed in nature and are found in bacteria, plants, and humans. They are essential to many physiological and biological processes.

Recent research revealed the potent inhibitory action of cysteine proteases by the compound 6,6'-dihydroxythiobinupharidine. The study findings reflected that in comparison to Cathepsin B, L, and papain (IC_{50} = 1359.4, 13.2 and 70.4 µM respectively), Cathepsin S exhibited the highest susceptibility to suppression by 6,6'-dihydroxythiobinupharidine (IC_{50} = 3.2 µM). M^{pro} of severe acute respiratory syndrome coronavirus 2 is not successively inhibited by 6,6'-dihydroxythiobinupharidine. Molecular docking revealed that Cathepsin S, L, and B's cysteine sulphur was fairly close to the thiaspirane ring of 6,6'-dihydroxythiobinupharidine, forming a disulfide bond which would provide stability to Cathepsins-DTBN complexes (Waidha K et al., 2021b).

4. Neothiobinupharidine and Thiobinupharidine

Neothiobinupharidine is a bioactive crystalline sulphur alkaloid with the chemical formula of $C_{30}H_{42}N_2O_2S$ which is same as that of neothiobinupharidine and can be synthesized as per the procedure in Jansen *et al*. (Figure 1) (Wrobel JT, 1967; Jansen DJ and Shenvi RA, 2013).

4.1 Immunosuppressive property

The immunosuppressive activity of thiobinupharidine and neothiobinupharidine was reported by Matsuda *et al*. in 2001. These dimeric sesquiterpene thioalkaloids, which lack hydroxyl groups, at a concentration of 1 µM, had a mild inhibitory effect on plaque development when incubated with splenocyte cultures containing SRBC for 4 days (Matsuda H et al., 2001).

4.2 Anti-metastatic effect

The metastatic effect of thiobinupharidine and neothiobinupharidine was reported by Matsuda *et al*. in 2003. When thiobinupharidine and neothiobinupharidine at 100 µM concentration were incubated with B16 melanoma cells showed inhibition of 22.7% and 28.3%. When the activity of these compounds were compared with the compounds containing 6-hydroxyl group, these compounds were considered as showing weak antimetastatic effect (Matsuda H et al., 2003).

4.3 Anti-cytotoxic property

The cytotoxic effect of neothiobinupharidine and thiobinupharidine was reported by Matsuda *et al*. in 2006. When thiobinupharidine and neothiobinupharidine at 10 µM concentration were incubated for 72 h with U937, B16F10 and HT1080 cells showed 10.9%, 4.4% −11.7% and 6.3%, 10.8%, −4.4%, respectively. When the activity of these compounds were compared with the compounds containing 6-hydroxyl group, these compounds were considered as showing weak cytotoxicity (Matsuda H et al., 2006). The cytotoxicity towards CEM/ADR5000 cells and CCRF-CEM cells was reported by Fukaya *et al*. in 2018. Both compounds showed stronger cytotoxicity on CEM/ADR5000 cells than doxorubicin with the degree of resistance of 1.65 µM

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and 0.92 µM, respectively. However, dose–response curves showed that thiobinupharidine was more efficacious against CCRF-CEM cells when compared with CEM/ADR5000 cells (Fukaya et al., 2018).

5. 6,6'-Dihydroxythionuphlutine B, 6-Hydroxythionuphlutine B, and Thionuphlutine B

The chemical formula of thionuphlutine B, 6-hydroxythionuphlutine B, 6,6'-dihydroxythionuphlutine B compounds are $C_{30}H_{42}N_2O_2S$, $C_{30}H_{42}N_2O_3S$, and $C_{30}H_{42}N_2O_4S$, respectively (Figure 1) (Wrobel JT, 1967).

5.1 Immunosuppressive property

The immunosuppressive effect of 6,6'-dihydroxythionuphlutine B, 6-hydroxythionuphlutine B and thionuphlutine B was reported by Matsuda *et al*. in 2001. 6-hydroxythionuphlutine B and 6,6'-dihydroxythionuphlutine suppressed the PFC formation at 1 µM (Matsuda H et al., 2001).

5.2 Anti-metastatic property

The anti-metastatic effect of thionuphlutine B and 6-hydroxythionuphlutine B was reported by Matsuda *et al*. in 2003. 6-hydroxythionuphlutine B, a dimeric sesquiterpene thioalkaloids containing the 6-hydroxyl group, showed an inhibition of 47.1% at 100 µM concentration against the penetration of B16 melanoma 4A5 cells ($p<0.01$). However, thionuphlutine did not show significant activity (Matsuda H et al., 2003).

5.3 Anti-cytotoxic property

The cytotoxic effect of thionuphlutine B and 6-hydroxythionuphlutine B was reported by Matsuda *et al.* in 2006. 6-hydroxythionuphlutine and thionuphlutine when incubated with U937, B16F10, and HT1080 cells for 72 hours at a concentration of 10 µM produced percentage inhibition of 94.1%, 99.1%, 98.7% and 7.8%, 2.2%, −12.0%, respectively. Thus it was proved that 6-hydroxythionuphlutine was cytotoxic compared to thionuphlutine (Matsuda H et al., 2006).

6. Nupharpumilamine

Nupharpumilamine is a novel thiaspiran sulfoxide type dimeric sesquiterpene alkaloid obtained from *N. Pumilum* (Figure 1). They classify as A, B, C, and D based on chemical and physicochemical evidence (Matsuda H et al., 2001; Matsuda H et al., 2003; Matsuda H et al., 2006; Matsuda H et al., 2019). No experiments were carried out in the previous decades on this compound. So, much information has not been found on this compound yet.

7. Nupharamine

Nupharamine has a chemical formula of $C_{15}H_{25}NO_2$ (Figure 1). It was obtained in *N. Japonica* and is used as a diuretic and treatment for stomach aches (Raghavan S and Rajendar S, 2016; Barluenga J et al., 1999). No experiments were carried out in the previous decades on this compound. So, much information has not been found on this compound yet.

CONCLUSION AND OUTLOOK

Nuphar alkaloids such as nupharamine, nupharpumilamine, nupharidine, 6-hydroxynupharidine, etc., are isolated from

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Nuphar plants such as *N. Lutea*, *N. Pumila*, and *N. Japonica*. Although these emerging alkaloids have been reported in cancer treatment, their use in other therapy areas remains unexplored. From the above studies it is clear that sulphur atom and 6-hydroxy group present in the ring system gives excellent activity to the compound such as cytotoxicity, immunosuppression etc. Our review indicated that very few studies have reported *in-vitro* and *in-vivo* tests on these compounds. Moreover, since Nuphar plants have been traditionally used for alleviating pain, as a tonic and digestive, we strongly believe that there are more uses for these compounds apart from cancer and what is researched in previous decades.

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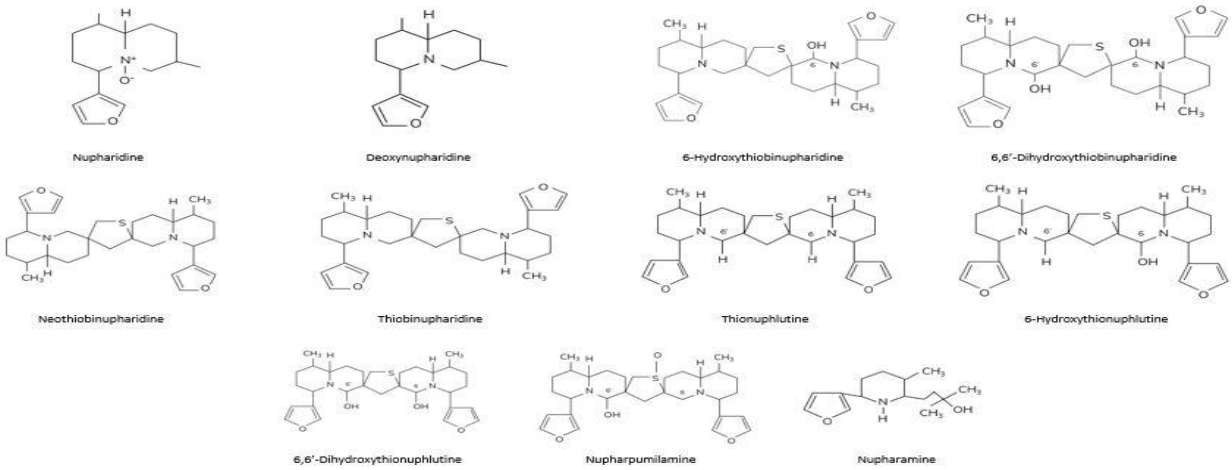
Figure 1 : Structures of various Nuphar alkaloids



Abbreviations: DNA: deoxyribonucleic acid; NF-κB: nuclear factor κB; PFC: plaque forming cell; ROS: reactive oxygen species

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