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ROLE OF NERIUM OLIENDER IN CANCER CELL

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ABSTRACT

Oleandrin is a monomeric compound extracted from leaves and seeds of Nerium oleander. It had been reported that oleandrin could effectively inhibit the growth of human cancer cells. However, the specific mechanisms of the oleandrin-induced anti-tumor effects remain largely unclear. Genomic instability is one of the main features of cancer cells; it can be the combined effect of DNA damage and tumour-specific DNA repair defects. DNA damage plays important roles during tumorigenesis. In fact, most of the current chemotherapy agents were designed to kill cancer cells by inducing DNA damage. In this study, we found that oleandrin was effective to induce apoptosis in cancer cells, and cause rapid DNA damage response, represented by nuclear RPA (Replication Protein A, a single strand DNA binding protein) and γH2AX(a marker for DNA double strand breaks) foci formation. Interestingly, expression of RAD51, a key protein involved in homologous recombination (HR), was suppressed while XRCC1 was up-regulated in oleandrin treated cancer cells.The compositions and methods disclosed may be useful for treating a variety of diseases or disorders including one or more cell-proliferative diseases or disorders, infections, and dementias.Cervical cancer is one of the most common gynaecological malignant tumors reported in women. Although a number of early screening and treatment options are available, mortality due to cervical cancer remains high. Nerium oleander L. is a potential medicinal plant that possesses a wide spectrum of pharmacological and physiological activities including anticancer activities.

INTRODUCTION

Nerium Oleander (N.olender)[1] is an evergreen shrub that is frequently grown as an ornamental plant in gardens and public areas.N.olender has linear and leatherty leaves that come in various colours, from dark green to grey green with distinct light yellowish vein.Its flowers are frugrant, funnel -shaped and arranged in clusters at the tip of twigs, withwhite to pink to deep red colour.The fruit is a narrow pod containing many silky haired seed. The plant is native to Mediterranean region of Africa and Europe.N.oleander is well known for its toxicity,as all parts of the plant contain numerous toxic compounds.The major toxic components are the cardiac glycosides oleandrin and neriin.Plant with red flower produce more cardiac glycosides that plant with white flowers, especially in the flowering stage and commonly, animal poisoning occur due to accidental contamination of food in some cases,due to consumption of toxic plants by hungry

animals.cardiac glycosides inactive the Na+/K+ATPase pump on the cytoplasm membrane of cancer cells. Oleander is a monomeric compoound extracted from leaves and seeds of neriumoleander,with a molecular weight of 576.73 and molecular formula of C32H48O9. As a kind of cardiacglycosides,it was well known for itseffect in treating congestive heart failure.Notonly that in the past decades,more and more studies have revealed that oleandrin can effectively inhibit proliferation of various cancer cells and induceapoptosis.Besides that oleandrin can also enhance the efficiency of radiotherapy.Interestingly the anti-tumor role of oleandrin seemed to be selective ,as oleandrin can kill certain humantumor cells but notmurine tumorcells.In a phase -1 study,oleandrinin a phase I study, oleandrin was used to remedy patients with refractory solid tumors, where oleandrin was found to be well tolerated and only few adverse events were reported. Up to now, various studies on many possible pathways have been made to elucidate the anti-cancer role of oleandrin. Some argues that oleandrin's ability to inhibit cancer cells proliferations were because of the decrease in levels of Na, K-ATPase. Mitochondrial injury caused by the generation of reactive oxygen species (ROS) was also taken into account. Others suggest that activation of caspase-3 by oleandrin may be a cause of tumor cells apoptosis. It had been reported that cancer cells were arrested in G2/M cell cycle by oleandrin, suggesting activation of DNA damage checkpoint. However, detailed mechanisms of the anti-tumor role of oleandrin are still not fully understood. As we know, Genomic instability is one of the main features of cancer cells, it can be the combined effect of DNA damage and tumour-specific DNA repair defects, and plays important roles during tumorigenesis. At the present, many chemotherapy agents were designed to target DNA damage repair to induce cancer cell apoptosis. Here, we investigated roles of oleandrin in induction of cancer cell apoptosis as well as its impact on DNA damage response. History (plant) Oleander is the common and species name for a poisonous evergreen shrub or small tree,

Fig:1 Nerium oleander in flower

Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order : Gentianales
Family: Apocynaceae
Genus: Nerium L.
Species: N. Oleander
Binomial Name: Nerium Oleander L.

Nerium oleander, in the dogbane family Apocynaceae, characterized by dark green, lanceolate leaves, flowers with a deeply 5-lobed corolla clustered at the end of the branches, and fruit in the form of a long, narrow capsule with numerous comose seeds. Oleander is one of the most poisonous plants to humans known. It is found from Portugal in Europe and Morocco in Northern Africa to China. Among other common names is rosebay.[2] the entire plant, including the milky white sap, is toxic, and any part can cause an adverse reaction. Even a small amount of ingestion can cause lethal or near lethal effects in humans, as well as many other animals, including horses, cattle, and other livestock. Despite its extremely high toxicity, oleander offers important ecological and aesthetic values. Economically, various animals can use it for food, such as the oleander caterpillar that feeds only on oleanders. For humans, the showy and often sweetly scented oleander flowers, which come in a variety of colors (white, red, pink, yellow, purple), are used for aesthetic purposes. The plants are used for ornamental purposes in parks, along roadsides, and in some U.S. states as a decorative freeway median, which deer will not consume.

Overview and description

Fig 2: Nerium oleander shrub

Oleander is a member of the Apocynaceae or dogbane family, a family of flowering plants in the Gentianales order that includes trees, shrubs, herbs,and lianas. Oleander, *Nerium oleander* is the only species currently classified in the genus *Nerium*.Oleander is a member of the Apocynaceae or dogbaneOleander grows to 2 to 6 meters (6.5 feet to 19.7 feet) tall, with spreading to erect branches. The leaves are in pairs or whorls of three, thick and leathery, dark green, narrow lanceolate, 5 to 21 centimeters (2-8 inches) long and 1 to 3.5 centimeters (0.4-1.4 inches) broad, and with an entire margin. The flowers grow in clusters at the end of each branch; they commonly are white, pink, red, yellow or purple, 2.5 to 5 centimeters (1-2 inches) in diameter, with a deeply 5-lobed corolla with a fringe round the central corolla tube[3]. They are often, but not always, sweetly scented. The fruit is a long narrow capsule 5 to 23 centimeters (2-9 inches) long, which splits open at maturity to release numerous down seeds.Oleander is native to a broad area from Morocco and Portugal eastward through the Mediterranean region and southern Asia to Yunnan in hisouthern parts of China . It typically occurs around dry stream beds.In the past, scented plants were sometimes treated as a distinct species *N. odorum*, but the character is not constant and it is no longer regarded as a separate taxon. Other common names for *N. oleander* include adelfa, alheliextranjero, baladre, espirradeira, florde São Jose, laurel de jardín, laurel rosa, Laurier rose, Flourier rose, olean, aiwa, rosa Francesca, rosa laurel, and rose-bay or rose bay (Laborde 1989)[4]. In Chinese it is known as*jiazhutaoe*taoeiv. The ancient city of Volubilis in Morocco took its name from the old Latin name for the flower.

Fig 3: (White,light pink,dark

METHODS

Preparation of *Nerium Oleander* Extract: The production of the *Nerium oleander* extract is generally similar to the hot water extraction technique. Hot water extraction provides *Nerium* species extracts which contain a variety of components including without limitation immunologically active polysaccharides. Preferred species of *Nerium* for preparation of extracts are *N. indicuim* and *N. oleander.* The term "plant matter"[5] denotes any part of the plant, although the less fibrous parts of the plant (branches, leaves flowers) are generally more useful than fibrous parts such as, for example, roots or lower, woody parts of stems. The extracts of the embodiments are preferably prepared from the branches, leaves and flowers of the *Nerium oleander* plant which can be sliced into pieces preferably ranging in size from about 2 cm to about 2.5 cm in length. Within about 1 week of collecting and slicing the plant material, the sliced plant material is suspended in a polar inorganic

solvent, such as water, and heated to about 100° C. Heating at about 100° C was continued for about 2.5 hours, during which time loss of liquid due to evaporation is compensated for by the addition of water to the vessel. At the end of the initial heat treatment, the density of the aqueous phase is determined. If the density is less than about 1010, the extract is again heated until the desired density is obtained. After the proper density is obtained, the mixture is allowed to cool to room temperature, filtered to remove large particulate matter, filtered again to eliminate small particulate matter, aliquoted into appropriate containers and sealed. After this second filtration, the sealed containers are again heated to about 100° C for about 1 hour.[6] Following this second heat treatment, the bottles are stored at room temperature for about 10 hours. In this form, the extract has a shelf life of about one year when stored between about 2° C and 4°C Cell Culture. **Combinational therapy:** MCF-7 cells were incubated with varying concentrations of GSH for 4 hours before the addition of NOE.[7] Cells were examined for apoptosis at 24 hours post treatment with GSH.Cell Viability Assay. MCF-7 cells incubated with GSH alone, NOE alone, or with GSH and NOE in combination were evaluated for cell survival using an MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. However, other routes of administration can be used. For example, absorption through the gastrointestinal tract can be accomplished by oral routes of administration, including but not limited to, ingestion, buccaland sublingual.

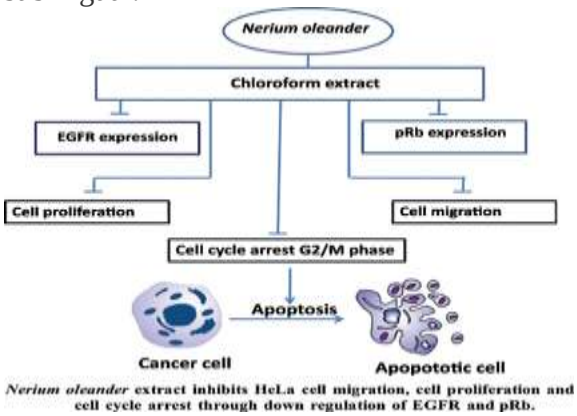

Fig 4: Chloroform extract of Nerium oleander

Example: Human breast cancer MCF-7 cells were cultured with GSH[9] alone (5 mM), NOE alone (50 µg/ml), or with 5 mM GSH and 50 µg/ml NOE in combination for 24 hours. The control sample contained neither GSH nor NOE. Therefore, as illustrated below in Table 1, the combination of NOE and GSH unexpectedly provided a highly synergistic effect with respect to apoptosis. These results suggest that NOE may be of potential value in the treatment of solid and/or immune resistant cancer.[10]

BRIEF DESCRIPTION OF THE DRAWINGS: The flow cytometry dot-plot[11] of a control showing MCF-7 cancer cells cultured in the absence of *Nerium oleander* extracts (NOE) and GSH. The frequency histogram of the data shown in showing the total population (18%) of apoptotic MCF-7 cancer cells (control). The flow cytometry dot-plot showing MCF-7 cancer cells cultured in the presence of 5 mM GSH. The frequency histogram of the data shown in showing the total population (20%) of apoptotic MCF-7 cancer cells. The flow cytometry dot-plot showing MCF-7[12] cancer cells cultured in the presence of 50 µg/ml NOE. The frequency histogram of the data shown in showing the total population (17%) of apoptotic MCF-7 cancer cells. The frequency histogram of the data shown in showing the total population (17%) of apoptotic MCF-7 cancer cells. The second embodiment flow cytometry dot-plot showing MCF-7 cancer cells cultured in the presence of 10 mM GSH and 50 µg/ml NOE. The frequency histogram of the data shown in showing the total population (77%) of apoptotic MCF-7 cancer cells.

Nerium oleander constituents: A pentacyclic triterpene, oleanderocioic acid, two flavonoid glycosides, such as quercetin and kaempferol, as well as cardenolide and oleandigoside were isolated from the leaves of *N. Oleander*. B-sitostero006C13 and oleanolic acid were isolated as the active components from flowers of the plant.

Toxic propertiesof N. oleander

The toxicity of *N. oleander* has been found for years. All parts of the plant especially seeds and roots contain cardiac glycosides.

TABLE 1: Cell apoptosis in 0-50µg/ml NOE and 0-5mm GSH

NOE (µg/ml)	GSH(mm)	%CELL survival	%CELL death	%CELL survival relativeto control	%CELL death relative to control
0	0	82	18	100	0
0	5	80	20	98	11
50	0	83	17	101	-6
50	5	38	62	46	244

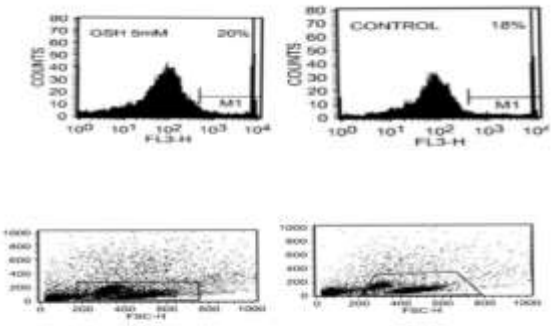

Fig 5: Therapeutic Nerium oleander extracts compositions (a)

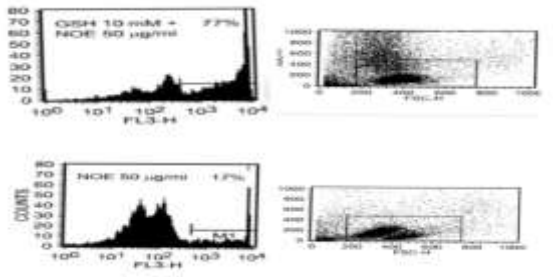

Fig 5: Therapeutic Nerium oleander extract compositions(b)

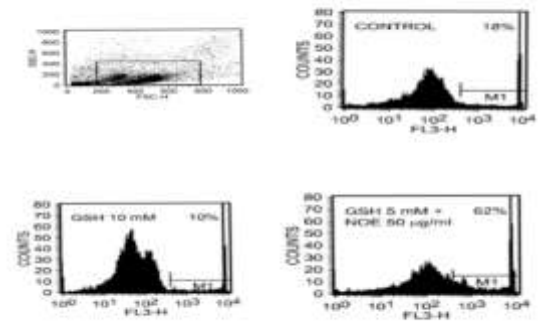

Fig 5: Therapeutic Nerium oleander extract compositions(c)

The structure of cardiac glycosides is similar to digitoxin of the foxglove plant. Several studies have indicated that *N. oleander* may act as insecticides, pesticides, rodenticides, and antimicrobial agents. Consumption of five *N. oleander* leaves can cause lethal poisoning. However, it was reported that one *N. oleander* leaf had severe toxiceffects in children. Controversially, ingestion of three leaves of *N. oleander* with a 7 years old child caused moderate poisoning with no complication. Mild toxicity was observed in an adult woman following consumption of five leaves of *N. Oleander*, without severe symptoms. Thus, determination of the fatal dose for *N. oleander* toxicity[14] has not fully understood and more studies should be done to found the lethal doses of the plant. The severity of *N.oleander* toxicity is related to several factors including the concentration of toxin in ingested part of the plant, age, and health condition of the subject who consumed the plant.

Toxic mechanism of N. oleander:

Cardiac glycosides component in *N. oleander* inhibits the "Na^+-K^+ ATPase pump" [15]in the membrane of cardiomyocytes, resulting in an increase in intracellular Na^+ concentration. Additionally, this increase changes the shift of Na^+-Ca^{2+} channels, resulting in an elevation in intracellular Ca^{2+} and contraction force and also cardiac automaticity. "Na^+-K^+ ATPase pump" inhibition changes the shift of K^+, resulting in increased level of K^+.[12] Hyperkalemia indicates the severity of toxicity in acute cardiac glycosides poisoning.

1.Toxic effects of N. oleander on lungs: Intramuscularly (IM) administration of *N. oleander* leaves extract (10 mL/kg) in both

hind limbs of rats showed mononuclear cell infiltrates in the lung tissue section, most frequently around the blood vessel 3, 12, and 24 h after administration. Dilation and even collapse in some alveoli were observed in alveolar tissue 24 h after administration. Massive infiltration along with hemorrhage and extravasation of blood cells and severe negative changes were also observed in the study group. Alveoli, alveolar sacs, and broncus were observed in section of the control lung tissue. The aqueous decoction of leaves extract of *N. oleander* leaves extract (10 mL/kg) induced histopathological changes in the Wistar rats lung tissues including alterations in the pulmonary tissue with disruption of bronchus mucosal folds[16]. Also, alveolar cells with extreme widening of lumen of the bronchiole and vascular lesions have been observed. Inflammatory cells, especially neutrophils, were frequently found in the bronchoalveolar region. In addition, lung sections of the control group showed normal histological architecture and numerous clear alveoli with thin interalveolar septa and alveolar sacs.

2 .Toxic effects of N. oleander on liver

The results of Prussian blue iron-stained sections after 3, 6, and 12 h of *N. oleander* leaves extract[17] (10 mL/kg, IM) administration showed extensive iron accumulation but in section after 12 h of administration, mild deposition in sinusoidal space was also observed particularly. Distinct bluish granules (ferritin) within hepatocytes 6 and 12 h after onset of acute phase response were observed. The extracts of *N. oleander* flowers (33 mg/kg, b.w.) induced hydropic degenerations in the liver tissue. In addition, mononuclear cell infiltration in the portal spaces with scattered necrosis of hepatocytes was induced by plant flower extract. Congestion and hemorrhage in some cases were also observed.

3. Toxic effects of N. oleander on heart:

Oral administration of 100 mg of *N. oleander* ethanolic extract showed diffuse mild interfascicularedema with congested vessels and many fragmentations of myofibrils in degenerated myocytes 14 days after treatment in heart muscles.[18] In addition, 200 mg of *N. oleander* ethanolic extract showed moderate interfascicularedema with dilated congested vessels and few degenerated myocytes with focal striation loss and focal vacuolar degeneration in the heart muscles; 30 days treatments animals with 100 mg of *N. oleander* showed focal mild interfascicularedema with congested vessels and very few degenerated myocytes in the heart muscles, while 200 mg of *N. oleander* showed focal marked interfascicularedema with congested vessels and moderately degenerated myocytes with vacuolationof the muscle.

Purported Uses and Benefits

To treat cancer

- Lab studies show some anticancer activity in cancer cell lines, but clinical trials to evaluate the anticancer activities of oleandrin in humans are lacking.

Scientific evidence is lacking to support the following claims:

- To treat congestive heart failure[19]
- To treat hepatitis
- To treat COVID-19
- To treat AIDS

Fig 6: Prevention and cure of disease

- Menstrual pain
- Skin problems,when applied to the skin.
- To treat Poison
- Asthma
- Seizures.

Fig 7: Different forms of oleander and uses

Side Effects

- Nausea[20], vomiting
- Diarrhea
- Fatigue
- Itching
- Pain at injection site
- Tumor pain
- Breast pain
- Abnormally high white blood cell counts fast and irregular heart rate

Case Report

Death of an adult diabetic man: Due to consumption of oleander leaves.

Death suspected from daily intramuscular injections: In a 43-year-old cancer patient, who used intramuscular injections of *Nerium oleander* extract for 2 months[21].

Accidental poisoning: In a woman who attempted to self-medicate for thyroid disease.

When taken by mouth: Oleander is likely unsafe. Consuming oleander leaf, oleander leaf tea, or oleander seed has led to deadly poisonings

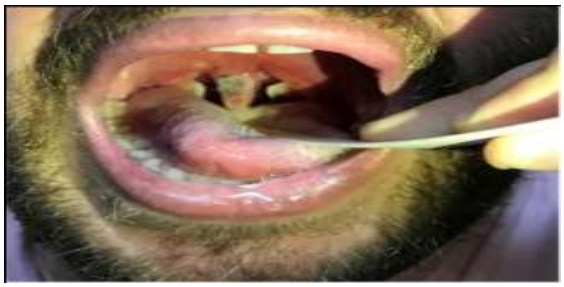

Fig 8: When applied through mouth

When applied to the skin: Oleander is possibly unsafe. It can be absorbed into the body and cause serious side effects. Touching oleander sap can cause a **rash**

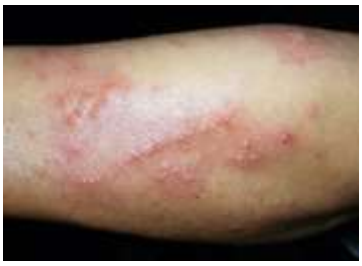

Fig 9: When applied through skin

Contraindication:

- Patients with hypercalcemia[22], hypokalemia, bradycardia, ventricular tachycardia, or heart failure should not use these products.
- These products should not be used outside of clinical trials.
- Oleander is no longer considered safe due to extreme toxicity.

Adverse Reactions

Common (Raw botanical) Consumption of *Nerium oleander* can be fatal . Onset of toxicity: Several hours after consumption. Symptoms include vomiting, abdominal pain, cyanosis, hypotension, hypothermia, vertigo, respiratory paralysis, and death. Can occur at serum oleandrin levels between 1.0 and 2.0 ng/mL. With Anvirzel™: Pain at injection site, fatigue, transient erythema, nausea, vomiting, and diarrhoea. With oral PBI-05204 extract:[23] Fatigue, nausea, diarrhea, arrhythmia .

Case Report:

Death of an adult diabetic man: Due to consumption of oleander leaves. Oleandrin levels in the blood were roughly 10 ng/ml.

Death suspected from daily intramuscular injections: In a 43-year-old patient[24] with metastatic synovial sarcoma of the knee who used daily intramuscular injections of *Nerium oleander* extract for 2 months. Symptoms included nausea, vomiting, severe stomach pain and bloating followed by a gradual reduction in liver enzymes and cardiopulmonary arrest.

Accidental poisoning: In a woman who attempted to self-medicate for thyroid disease.

Drug Interaction:

Antibiotics (Macrolide antibiotics) Interaction Rating: **Major** Do not take this combination.

Oleander can affect the heart, Some antibiotics might increase how much oleander the body absorbs. Increasing how much oleander the body absorbs might increase the effects and side effects of oleander.

Some antibiotics called macrolide antibiotics[25] include **erythromycin**, azithromycin, and clarithromycin.

Antibiotics (Tetracycline antibiotics) Interaction Rating: **Major** Do not takes this combination. Taking some antibiotics called tetracycline antibiotics along with oleander might increase the chance of side effects from oleander.

Digoxin (Lanoxin) Interaction Rating: **Major** Do not take this combination.

Digoxin (Lanoxin) helps the heart beat more strongly. Oleander also seems to affect the heart. Taking oleander along with digoxin can increase the effects of digoxin and increase the risk of side effects[26]. Do not take oleander if you are taking digoxin (Lanoxin) without talking to your health care professional.

QuinineinteractionRating: **Major** Do not take this combination.

Oleander can affect the heart. Quinine can also affect the heart. Taking quinine along with oleander might cause serious heart problems.

Laxative Stimulant interaction. Rating: **Major** Do not take this combination.

Oleander can affect the heart. The heart uses potassium. Laxatives called stimulant laxatives can decrease potassium levels in the body. Low potassium levels can increase the chance of side effects from taking oleander.

Some stimulant laxatives include bisacodyl (Correctol, Dulcolax), cascara, castor oil (Purge), senna (Senokot), and others.

Water pills (Diuretic drugs)

Interaction Rating: **Major** Do not takes this combination. Oleander might affect the heart. "Water pills"[27] can decrease potassium in the body. Low potassium levels can also affect the heart and increase the risk of side effects from oleander.

Some "water pills" that can deplete potassium include chlorothiazide (**Diuril**), chlorthalidone (**Thalitone**), **furosemide** (**Lasix**), hydrochlorothiazide (HCTZ, HydroDiuril, **Microzide**), and others.

Precautions:

When taken by mouth: Oleander is likely unsafe. Consuming oleander leaf, oleander leaf tea, or oleanderseed hasledtodeadlypoisonings.

When applied to the skin: Oleander is possibly unsafe. It can be absorbed into the body and cause serious side effects. Touching oleander sap can cause a **rash**.

Pregnancy and breast-feeding: Taking oleander by **mouth** is likely unsafe while pregnant or breast-feeding. It might cause an **abortion** or cause birth defects[28]. There isn't enough reliable information to know if oleander issafetoapplytothe **skin**. Stay on the safe side and avoid use.

Children: Oleander is likely unsafe when taken by mouth. Taking the oleander leaf, oleander leaf tea, or oleander seed has led to deadly poisonings.

Treatment

Oleandrin Induces cell death in multiple cancer cell lines:

To better understand however oleandrin induces cancer cell apoptosis, A549 cells[29] were treated with oleandrin, followed by detection of apoptosis by flow cytometry (FCM). Increased concentrations of oleandrin (0.01ug/ml, 0.02ug/ml, 0.04ug/ml) were incubated with A549 cells for 24 hours, where as low as 0.02 ug/ml was found to be sufficient in apoptosis induction . Compared with the control group, apoptosis of A549 cells with treatment of oleandrin (0.02ug/ml,0.04ug/ml) groups showed a statistically significant increase. Furthermore, apoptosis was induced by oleandrin (0.02ug/ml) in a time-dependent manner . Similar experiments were performed in additional two cell lines, including HBE (a human bronchial epithelial cell line) and H1299 (a human non-small cell lung carcinoma cell line). Interestingly, the two cancer cell lines were more sensitive to oleandrin treatment, while HBE cells showed the little the toxicity.

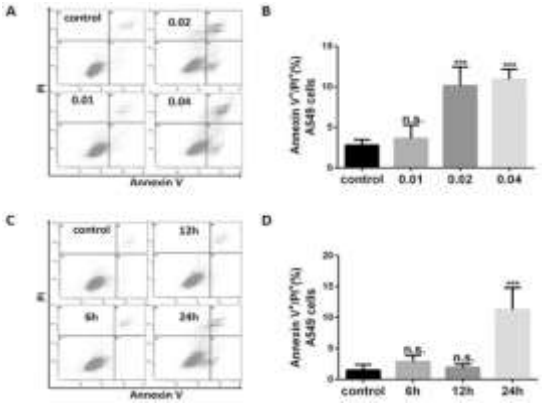

Fig 10: Toxicity of HBE cells

Oleandrin induces DNA damage response in lung cancer cells

RPA is a ubiquitous eukaryotic single-stranded DNA[30] (ssDNA) binding protein that serves to protect every generated ssDNA from degradation . With the method of immunofluorescence, we measured localization of RPA in A549 and H1299 cells following treatment with oleandrin (0.02 ug/ml). It was obvious that oleandrin could significantly increase levels of RPA foci formation at 12 hours , at which point no significant cell death was yet detected .

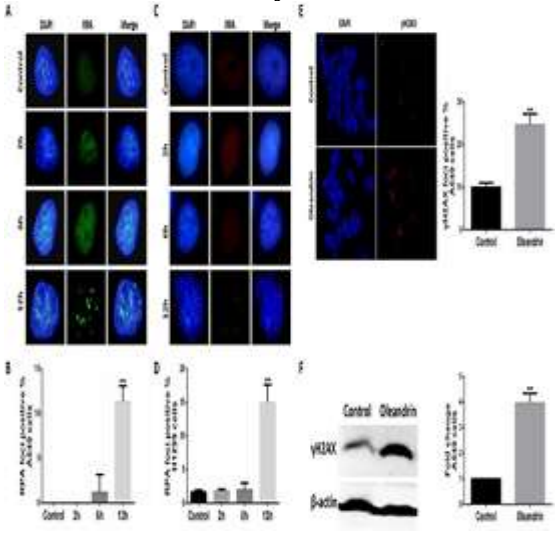

Fig 11: Toxicity of lung cancer cells

Oleandrin down-regulates expression of HR protein RAD51

There are two major DSB repair pathways in higher eukaryotes, HR and non-homologous end joining (NHEJ). And, HR plays an important role in DSB repair. To ensure whether the DNA breaks-induced by oleandrin were repaired by HR pathway. We measured the expression of RAD51 by western blot[31]. In fact, the expression level of RAD51 reduced significantly in A54and H1299 cells following treatment with oleandrin.

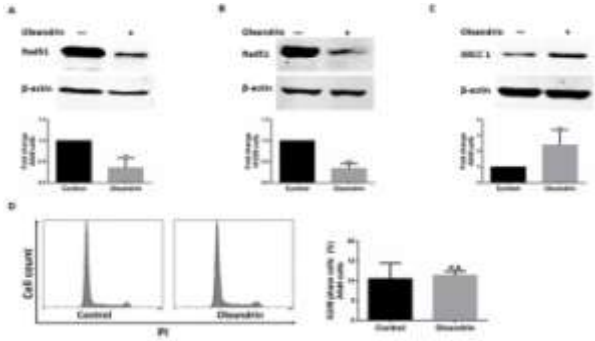

Fig 12: Expression of RAD51

Cases:

There were **18 deaths** [32](mortality rate 17.65%), most of them (82.55%) occurring within 24 hours of intake. There was a striking female preponderance both in the incidence of symptomatic oleander toxicity as well as mortality. The female mortality rate was 23.41%, whereas males scored only 10.91%.

42-year-old woman with a known history of malign mesenchymal cancer was admitted to the emergency department with digoxin-like toxicity of 4 h duration.

In one study of 300 yellow oleander seed ingestions[33] with suicidal intent,12%of the patients had palpitations, while 46% had some type of arrhythmia;sinus bradycardia was present in 49% of the patients,and ischemic electrocardiogram changes were noted in 399 cases.

Conclusion

In conclusion, oleandrin could effectively induce the death of cancer cells[34] at a very low concentration; the anti-tumor role of oleandrin may be related with DNA damage repair; and oleandrin may be a novel HR inhibitor by suppressing the expression of Rad51. The extraction of Nerium oleander L.contains potential bioactive compounds that inhibite HELA CELLS[35] proliferation,cell migration and arrest cell cycle at the G2/M.pha**se**

REFERENCES:

1. Rania H.Abdou, WalaaA.Basha,and Waleed F.khail. Subacute Toxicity of Nerium oleander Ethanolic extract in

mice.ToxicologicalResearch-Korea society of Toxicology. Toxicology Research.2019 jul;35(3):233-239 published online 2019 jul 15.

2. Kjeldsen K, Norgaard A and Gheorghiade M. Myocardial Na,K-ATPase:The molecular basic for the hemodynamic effect of digoxin therapy in congestive heart failure,cardiovascular research. 2002;55:710-713.
3. Lin Y, Ho DH, Newman RA. Human tumor cell sensitivity to oleandrin is dependent on relative expression of Na+, K+-ATPase subunitst. Journal of experimental therapeutics & oncology. 2009;8:271–286.
4. Nasu S, Milas L, Kawabe S, Raju U, Newman RA. Enhancement of radiotherapy by oleandrin is a caspase-3 dependent process. Cancer letters. 2002;185:145–151.
5. McConkey DJ, Lin Y, Nutt LK, Ozel HZ, Newman RA. Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer research. 2000;60:3807–3812.
6. Nasu S, Milas L, Kawabe S, Raju U, Newman RA. Enhancement of radiotherapy by oleandrin is a caspase-3 dependent process. Cancer letters. 2002;185:145–151.
7. Pathak S, Multani AS, Narayan S, Kumar V, Newman RA. Anvirzel, an extract of Nerium oleander, induces cell death in human but not murine cancer cells. Anti-cancer drugs. 2000;11:455–463.
8. Raghavendra PB, Sreenivasan Y, Manna SK. Oleandrin induces apoptosis in human, but not in murine cells:dephosphorylation of Akt,expression of FasL,and alteration of membrane fluidity.Molecular immunology.2007;44:2292-2302.
9. Yang P, Menter DG, Cartwright C, Chan D, Dixon S, Suraokar M, Mendoza G, Llansa Newman RA. Oleandrin-mediated inhibition of human tumor cell proliferation: Importance of Na, K-ATPase α subunits as drug targets. Molecular cancer therapeutics. 2009;8:2319–2328.
10. Newman RA, Yang P, Hittelman WN, Lu T, Ho DH, Ni D, Chan D, Vijjeswarapu M, Cartwright C, Dixon S. Oleandrin-mediated oxidative stress in human melanoma cells. Journal of Experimental Therapeutic Oncology. 2006;5:167–181.
11. Newman RA, Kondo Y, Yokoyama T, Dixon S, Cartwright C, Chan D, Johansen M, Yang P. Autophagic cell death of human pancreatic tumor cells mediated by oleandrin, a lipid-soluble cardiac glycoside. Integrative cancer therapies. 2007;6:354–364.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation.Cell. 2011;144:646–674
13. ZhengqiangBao, Baoping Tian, and Songmin Ying.Oleandrin induces DNA damage responses in cancer cells by suppressing the expression of Rad51.
14. Chen J, Le S, Basu A, Chazin WJ, Yan J. Mechanochemical regulations of RPA's binding to ssDNA. Sci Rep. 2015;5:9296
15. Leaf Extract of *Nerium oleander* L. Inhibits Cell Proliferation, Migration and Arrest of Cell Cycle at G2/M Phase in HeLa Cervical Cancer Cell.
16. ShubhasmitaMohapatra et al. Anticancer Agents Medical Chemistry. 2021. Copyright© Bentham Science Publishers; For any queries.
17. Desai, U. R. 2000. Cardiacglycosides, *Virginia Commonwealth University School of Pharmacy*. Retrieved December 11, 2008.
18. Erwin, V. den Enden. 2004. *Illustrated Lecture Notes on Tropical Medicine.* Prince Leopold Institute of Tropical Medicine. Retrieved December 11, 2008.

19. Royal Botanic Garden Edinburgh. Flora Europaea (FE). N.D. Nerium oleander L.Retrieved December 11, 2008.
20. Flora of China (FOC). n.d. *Nerium oleander L.*. *FOC* 16: 173. Retrieved December 11, 2008.
21. Goetz, R. J., T. N. Jordan, J. W. McCain, and N. Y. Su. 1998.India plants poisonous to livestock and pets. Cooperative Extension Service, Purdue University. Retrieved December 11, 2008.
22. Huxley, A., M. Griffiths, and M. Levy (eds.). 1992. *The New RHS Dictionary of Gardening*. Macmillan. ISBN 033474945.
23. Inventors: Mamdooh Ghoneum, Los AngSeles, CA (US) ; Huseyin Ziya Ozel, Gayrettepe (TR). Therapeutic Nerium Oleander Extract Compositions And Methods Of Using .
24. This application is a continuation of U.S. patent application Ser. No. 12/218,134 filed Jul. 9, 2008 and claims the benefit of U.S. Provisional Application No. 60/959,028 filed on Jul. 9, 2007. Patent No.: US 8,486,465 B1
25. Lin Y, Ho DH, Newman RA. Human tumor cell sensitivity to oleandrin is depend on relative expression of $Na^{+}Ka^{+}$-ATP ase subunits.Date of Patent Jul. 16,2013.Experimental Therapeutic Oncology. 2010;8(4):271-286.
26. Manna SK, Sah NK, Newman RA, et al. Oleandrin suppresses activation of nuclear transcription factor-kappaB, activator protein-1,and c-Jun NH_2-terminal kinase.
27. Afaq F, Saleem M, Aziz MH, et al. Inhibition of 12-0-tetradecanoylphorbol-13-acetate-induced tumor promotion markers in CD-1 mouse skin by oleandrin. Toxicological Application Pharmacology. Mar 15 2004;195(3):361-369.
28. Shubhasmita,Mohapatra et al. Anticancer Agents Med Chem. 2021.Leaf Extract of *Nerium oleander* L. Inhibits Cell Proliferation, Migration and Arrest of Cell Cycle at G2/M Phase in HeLa Cervical Cancer Cell.
29. Behçet Al, PınarYarbil, and CumaYıldırım.A Case of non –fatal Oleander poisioning.
30. Kakrani, A. L., Rajput, C. S., Khandare, S. K., and Redkar, V. E. Yellow oleander seed poisoning with cardiotoxicity. A case report. Indian Heart J 1981;33(1):31-33.
31. Kaojarern, S., Sukhupunyarak, S., and Mokkhavesa, C. Oleander Yee tho poisoning. Journal Medical Association .Thai. 1986;69(2):108-112.
32. Mallick, B. K. Cardiotoxicity in yellow oleander seed poisoning. Journal of Indian Medical Association. 1984;82(8):296-297.
33. Manna, S. K., Sah, N. K., Newman, R. A., Cisneros, A., and Aggarwal, B. B. Oleandrin suppresses activation of nuclear transcription factor-kappaB, activator protein-1, and c-Jun NH2-terminal kinase. Cancer Research. 7-15-2000;60(14):3838-3847.
34. McConkey, D. J., Lin, Y., Nutt, L. K., Ozel, H. Z., and Newman, R. A. Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Research. 7-15-2000;60(14):3807-3812.
35. Mekhail, T., Kaur, H., Ganapathi, R., Budd, G. T., Elson, P., and Bukowski, R. M. Phase 1 trial of Anvirzel in patients with refractory solid tumors. Invest New Drugs 2006;24(5):423-427.