International Journal in Physical and Applied Sciences
Volume 06 Issue 06, June 2019 ISSN: 2394-5710 Impact Factor: 4.657
Journal Homepage: http://ijmr.net.in, Email: irjmss@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

"ROLE OF ISOFLAVONES IN DRUG DELIVERY WITH SPECIAL REFERENCE TO CHICKEN EGG LYSOZYME ON ASCITE CARCINOMA CELL: EXPERIENCED BASED RESEARCH"

Badhei Sanjukta, Dutta Amit Kumar and Thakur Piyush Kumar
School of Biological and Chemical Sciences,
MATS University, Raipur, Chhattisgarh-492002

ABSTRACT

Drug delivery is the formulations, technologies and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. Dietary polyphenols are very essential and are widely studied due to their various biological activities like anti-cancerous, antibacterial, anti inflammatory, anti diabetic etc. Lysozyme is an enzyme having antibacterial property which is found in various types of birds, mammals and insects. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. The treatment of cancer is complicated in that the drugs used target human cells, albeit cells that have undergone genetic changes and are dividing at a fast and uncontrolled rate. Frequently occurring secondary cancers associated with anticancer drug therapy are myelodysplastic syndrome and acute leukemias, risk of which is increased particularly with the use of alkylating agents and topoisomerase inhibitors. Flavonoids play an important functional role in medicine preparation with herbal and insect components. Current research involving liposomes is focused on improving the delivery of anticancer drugs. The histopathology studies revealed only mild hepatotoxicity and nephrotoxicity when compared to the normal and standard. The splenic cellularity also did not show much variation from normal. At a prime dose of 100 mg has shown promising anticancer activity in vivo against Ascite Carcinoma when compared to standard drug with minimum toxic effects.

Key word:Isoflavones, Swiss albino, Drug Analysis, Carcinoma, Tissue Sectioning

INTRODUCTION

DRUG:

Drug is a chemical substance which is consumed for temporary physiological change in the body[1]. It is also known as medicine which is used to cure, prevent or diagnose a disease and are used for limited duration on a regular basis for chronic disorders[2]. Drugs can be classified according to chemical structure,binding to same biological target etc.The process in which new medications are discovered is known as drug discovery. In ancient time drug were discovered by identifying the active ingredients from traditional remedies. Themodern drug discovery involves various processes. Those are identification of screening hits,medicinal chemistry and optimization of those hits to increase the affinity, selectivity, potency,metabolic stability & oral bioavailability. Once the

compound fulfills all of these requirements then the compound got identified and then proceeds for drug development. Discovering drug may be a commercial success or public health success involving a complex interaction between investor,industry, academia patent laws, regulatory exclusivity, marketing etc[3].Drug delivery is the process in which a pharmaceutical compound is formulated, technologies used and systems for transporting in the body to achieve the desired therapeutic effect which includes systemic pharmacokinetics. It is concerned with both quantity and duration of drug presence. Drug delivery is the concept of heavily integrated with dosage form and route of administration[4].

The modification of drug release profile, absorption, distribution and elimination are done by Drug delivery technologies to improve the efficacy of product and safety. It is also patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. The most common routes of administration are non-invasive per oral topical, transmucosal and inhalation routes.
Some medications likepeptide and protein, antibody, vaccine and gene based drugs may not delivered by using these routes. Because of susceptibility towards enzymatic degradation or efficiently they can't be absorbed into the systemic circulation because of molecular size and charge issues to get the therapeutic effect. Due to this reason many protein and peptide drugs have to be delivered through injection or a nano-needle array. For example, many immunizations are based on the delivery of protein drugs and are often done by injection[5-6].

Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues), sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation, and methods to increase survival of peroral agents which must pass through the stomach's acidic environment. In order to achieve efficient targeted delivery, the designed system must avoid the host's defense mechanisms and circulate to its intended site of action[4,7-9]. Flavonoids play an important functional role in medical preparation with herbal and insect components. Natural flavonoids that have been purified inhibit specific enzymes to stimulate some hormones and neurotransmitters. Flavonoids are classified into seven groups (Flavanones, flavones, flavonols, isoflavone, chalcones aurones and proanthocyanidins). Many types of enzymes are inhibited by several classes of the flavonoids. The inhibition may be competitive but more often it is allosteric inhibition which may cause architectural changes in the active site of the target enzyme[9-10].

International Journal in Physical and Applied Sciences
Volume 06 Issue 06, June 2019 ISSN: 2394-5710 Impact Factor: 4.657
Journal Homepage: http://ijmr.net.in, Email: irjmss@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

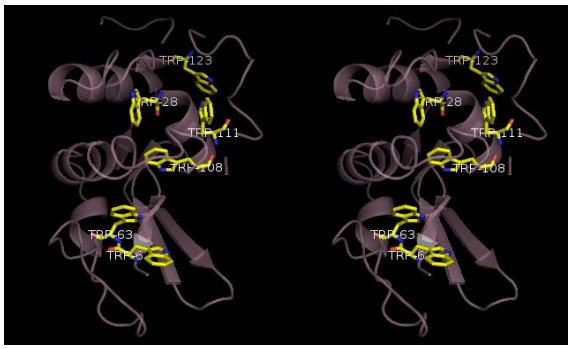


Figure showing cross-eye representation of Chicken Egg Lysozyme(PDB ID: 6lyz)

BIOLOGICAL MACROMOLECULE: CHICKEN EGG WHITE LYSOZYME

Lysozyme is an enzyme having antibacterial property which is found in various types of birds, mammals and insects. It has various pharmaceutical and pharmacological properties like anti-tumor, anti-viral, anti histamic, anti- inflammatory, immune modulatory properties. Lysozyme has the capacity to cure various diseases by binding with various drugs and realizing them in their target site. Lysozyme has the capacity to carry drug, so it has important role in medicinal point of view. Hence the study on lysozyme and ligand interaction is very important[11-13].

FLAVONOIDS: BIOMEDICINAL ASPECTS:

Flavonoids constitute the largest family of polyphenols. It is characterized by diphenylpropane skeleton ($C_6C_3C_6$), in which two aromatic rings are linked by three carbons by forming a heterocyclic ring. These molecules are easily available in human diet. The intake of flavonoids decreases the chances of various diseases like cancer, cardiovascular diseases, stroke, because these compounds have radical scavenging, anti-inflammatory, anti-cancer, anti-tumor, anti-oxidative properties. Flavonoids have two bands in UV visible spectra, one is at 240-280 due to benzoyl moiety and other is due to cinnamoyl moiety at 300-400nm[1,7,9].

CANCER:

Cancer is a group of diseases involving abnormal cell growth with the potential to spread to other parts of the body. Some contrast which do not spread to other parts of the body, with benign tumors. A lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements are the possible signs and symptoms and these symptoms may indicate cancer, they may have other causes. There are around 100 types of cancers affect humans.Cancers are classified on the basis of type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. Carcinoma develops from the epithelial tissue[14]. Specifically it begins in a tissue which lines the inner or outer surface of the body and that arises from cell originating in the endodermal, mesodermal or ectodermal germ layer during embryogenesis. Breast Cancer(BC) is the most commonly diagnosed cancer in the world and remains the leading cause of cancer-related death in women.

ANTICANCER:

Any drug that is effective in the treatment of malignant, or cancerous, diseaseis anticancer drug which is also known as antineoplastic drug. Anticancer drugs can be classified as; these include alkalyting agents, anti metabolites, natural products, and hormones. Apart from these there are a number of drugs which show anticancer activity. Thus those are used in the treatment of malignant disease. Most anticancer drugs are administered intravenously; however, some can be taken orally, and others can be injected intramuscularly or intrathecally (Within the spinal cord).

The treatment of cancer is complicated in that the drugs used target human cells, albeit cells that have undergone genetic changes and are dividing at a fast and uncontrolled rate. However, certain anticancer drugs can differentiate to some degree between normal tissue cells and cancer cells, and the rate at which cancer cells proliferate may in fact play a role in the apparent selectivity of agents. In the late 20th and early 21st centuries, the identification of molecular features unique to cancer cells fueled the development of targeted cancer therapies, which possess a relatively high degree of specificity for cancer cells. In rare instances prolonged use of anticancer drugs can lead to the development of secondary cancers. The type of agent, the primary cancer that it is used to treat, and the total cumulative dose administered influences the extent to which an anticancer drug is carcinogenic (cancer-causing). Frequently occurring secondary cancers associated with anticancer drug therapy are myelodysplastic syndrome and acute leukemias, risk of which is increased particularly with the use of alkylating agents and topoisomerase inhibitors[14-15].

MATERIALS& METHODOLOGY

All the chemicals used in the present study were from Sigma–Aldrich, USA. Adult female Swiss albino mice of 6–8 weeks old weighing 25–30g, inbred at Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, India were used throughout the study. The laboratory mouse is a small mammal which is bred and used for scientific research. Laboratory mice are usually of the species Swiss albino. They are the most commonly used mammalian research model and are used for research in genetics, psychology, medicine and other scientific disciplines[16].

Animals were housed in polypropylene cages containing sterile paddy husk as bedding material under hygienic conditions with a maximum of four animals in a cage. They were maintained under controlled conditions (10:14 h light: dark), temperature (23 ± 3 °C). Animals were fed on autoclaved standard mice food pellets (Hindustan Lever) and water ad libitum. The animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee (IAEC)[17].

EXPERIMENTAL ANIMAL:

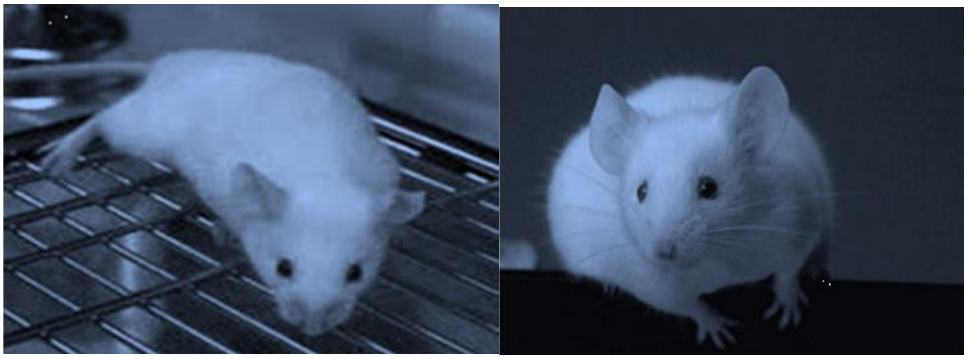


Figure Showing the Swiss albino mice for Experimental purpose

Testing of anticancer activitycan be done by cancer cell line induced in the mice. After that, the crude drug will be feeded to the experimental mice followed by the Histopathological Analysis.

RESULTS &DISCUSSION

In vivoAnticancer Studies

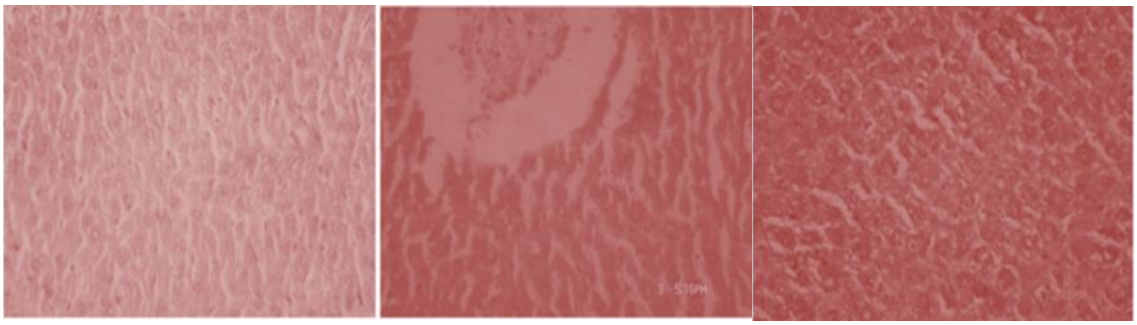
The drug solutions were prepared daily just prior to the injection by suspending them in 0.25% CMC (carboxy methyl cellulose) and was administered intraperitoneally. The dose of the standard drug, Isoflavones was selected as 3.5 mg/kg mice body weight. This was calculated from the human dose using an appropriate conversion factor. Carcinoma cells to induce cancer in animal model (mice) were obtained and the cells were maintained as ascites tumor in Swiss albino mice by intraperitoneal inoculation of 1×10^6 viable cells. Acute toxicity studies were carried out as per the OECD guidelines, 2001. For determining the maximum tolerated dose, the standard protocol was followed by administering to normal Swiss albino mice at the dose range up to 2000 mg/kg body weight after depriving them of food for 18 h. Animals were observed for any symptoms of toxicity continuously for 4 h, then after 24 h and finally the number of survivors were noted after a period of 72 h. Depending on the results obtained, the therapeutic doses for further studies were selected (1/10th to 1/20th of the maximum tolerated dose)[16-17].

In vivoToxicological Studies

On day 15, blood samples from six animals in each group were taken in microfuge tubes containing EDTA for hematological studies and the serum was collected for biochemical studies. The animals were then sacrificed by cervical dislocation, dissection was done and the liver, kidney and spleen were removed for histopathology studies[16-17].

Histopathology of Liver

Animals were sacrificed on day 15 and histopathology of liver was studied for signs of hepatotoxicity. The histology of liver of standard group showed cellular infiltration, congestion and mild central vein dilatation. Isoflavones(IFV-3@50 mg/Kg) treated group showed only mild central vein dilation suggesting less hepatotoxicity compared to control and standard.

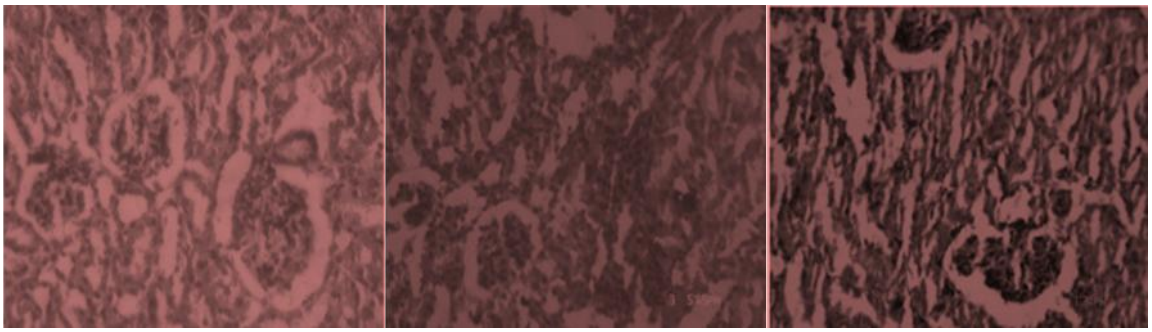


NORMAL CONTROL IFV-3(50mg/Kg)

Micro Photographs showing the histopathology of Liver of Swiss albino mice of 3 groups

Histopathology of Kidney

Animals were sacrificed on day 15 and histopathology of kidney was studied for signs of nephrotoxicity. The standard showed signs of nephrotoxicity due to glomerular infiltration. The Isoflavones(IFV-3@100 mg/Kg) treated group also showed mild glomerular infiltration. There were no signs of tubular necrosis, casts and glomerular congestion, which was indicative of only mild nephrotoxicity with Isoflavones(IFV-3@100 mg/Kg).



NORMAL CONTROL IFV-3(100mg/Kg)

Micro Photographs showing the histopathology of Kidney of Swiss albino mice of 3 groups

Histopathology of Spleen

Animals were sacrificed on day 15 and tissue sections of spleen were studied for signs of spleentoxicity. Mild loss in spleen architecture is observed in Isoflavones(IFV-3@50 mg/Kg) treated mice spleen[16-17].

NORMAL CONTROL IFV-3(100mg/Kg)

Micro Photographs showing the histopathology of Spleen of Swiss albino mice of 3 groups

CONCLUSION

Molecular Docking

Recently molecular docking becomes an important tool to investigate the protein–ligand binding study. The docking poses of diadzein and genistein in lysozyme are represented and for discussion we have chosen the first docked conformation in each case because it posses the minimum energy. It has been found that diadzein and genistein bind within H-bonding distance to the tryptophan residues (Trp 62 and Trp 63) of lysozyme. But the distance of diadzein is found greater (3-O to Ne, Trp 63: 10.7 A°; 4-C=O to Ne, Trp 62: 16.1 A°) than genistein (3'-O to Ne, Trp 63: 6.9 A°; 4-C=O to Ne, Trp 62: 13.8 A°). The expected interaction of the ligands with two tryptophan residues, Trp 62 and Trp 63 can also be explained on the basis of observed quenching of fluorescence intensity of lysozyme by the polyphenols. The distance of genistein (4'-O) to Trp 108 is found to be 13.2 A° and that is for diadzein (2'-O to Ne, Trp 108) is 16.7 A°. According to the FRET results it has also been found that genistein is present in the close vicinity to lysozyme than diadzein molecule. To recognize the residues involved in the binding, we have estimated the accessible surface area (ASA) of residues of native and complexed protein. No change in ASA for Trp 62 and Trp 63 are observed. The participation of the Trp62 and Trp63 residues (within H-bonding distance) in the interface reveals that ligands are looking for the active site of the protein. Both these moieties (Trp62 and Trp63) are situated in active site of the protein and vital for enzymatic action.

The interactions of dietary flavonoids genistein and diadzein with chicken egg lysozyme have been executed using CD and molecular docking studies. The Kb value of genistein is found greater than

diadzein towardslysozyme. The energy transfer parameters for the binding are calculated and it is observed that there is a chance of energy transfer from donor (lysozyme) to the acceptors (genistein and diadzein). CD results indicate that the polyphenols are capable to increase the helical content of lysozyme during binding process. Molecular docking study has been performed to substantiate the experimental facts. It has been observed that both the ligands bind near to Trp 62 and Trp 63, but the genistein binds closely than diadzein. Binding free energies obtained from docking results and PEARLS are in good correlation with each other for the protein– ligand complexes. The binding of dietary polyphenols with lysozyme at the molecular level suggests a further insight into the success of the drugs in pharmaceutics.

Drug Delivery & Histopathology

In the light of observations, it can be concluded that Isoflavones, at a dose of 100 mg/kg, optimally inhibits the growth of EAC cells in vivo. This is evident from the reduced tumor weight and enhanced life span of that study group. The treatment with Isoflavones (100mg/Kg) restored the deviated hematological and biochemical parameters to the normal range. It is an effective antineoplastic agent with comparatively less toxic effects. It is necessary that the anti-tumor activity of this Schiff base should be carried out against other tumor cell lines which may bring promising results in cancer therapy.Current research concluded that liposomes are focused on improving the delivery of anticancer drugs. The histopathology studies revealed only mild hepatotoxicity and nephrotoxicity when compared to the normal and standard. The spleenic cellularity also did not show much variation from normal. At a prime dose of 100 mg has shown promising anticancer activity in vivo against Ascite Carcinoma when compared to standard drug with minimum toxic effects.

ACKNOWLEDGEMENT

We would like to thanks our Hon'ble Vice Chancellor Sir & Respected Registrar Sir of MATS University, Raipur(CG) for giving us permission to carry out research in the School of Biological and Chemical Sciences. Our deep gratitude towards the Hon'ble Director General Sir of Chhattisgarh Council of Science & Technology, Raipur for giving us the facility to perform our Drug Delivery Animal Study. Our sincere thanks to Prof.(Dr.) Kallol Kumar Ghosh, Professor, SOS in Chemistry, Pt. Ravishankar Shukla University, Raipur(CG) for his all time help, support and guidance towards this research work.

REFERENCES

1. Falcone ML, Rius SP, Casati P(2012). Flavonoids: Biosynthesis. Biological Functions and Biotechnology applications.Frontiers in Plant Science;3: 222-237.
2. Havsteen BH (2002). The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics;96: 67-202.

3. Wang, NX.; von Recum, HA(2011). Affinity-Based Drug Delivery. Macromol Biosci. 11: 321–332.

4. Bertrand N, Leroux JC(2011). The journey of a drug carrier in the body: an anatomo-physiological perspective. Journal of Controlled Release; 161(2): 152– 163.

5. Kaufman PB, Duke JA, Brielmann H, Boik J, Hoyt JE(1997). A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: implications for human nutrition and health. J Altern Complement Med.; 3(1): 7–12.

6. Heber D(2008). Berdanier, C.D; Dwyer, J.T.; Feldman, E.B., eds. Plant Foods and Phytochemicals in Human Health. CRC Press; 1: 176–181.

7. Hsieh CY., Santell RC., Haslam SZ., Helferich WG.(1998). Estrogenic effects of genistein on the growth of estrogen receptorpositive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res.;58: 3833–3838.

8. Di X., Yu L, Moore AB, Castro L., Zheng X., HermonT., Dixon D(2008). A low concentration of genistein induces estrogenreceptor-R and insulin-like growth factor-I receptor interactions andproliferation in uterine leiomyoma cells. Hum. Reprod; 23:1873–1883.

9. Jiang X., Patterson NM, Ling Y., Xie J, Helferich, WG.,Shapiro DJ(2008). Low concentrations of the soy phytoestrogen genisteininduce proteinase inhibitor 9 and block killing of breast cancer cells byimmune cells. Endocrinology;149: 5366–5373.

10. Ju YH., Allred CD., Allred KF., Karko KL., Doerge DR.,Helferich WG(2001). Physiological concentrations of dietary genistein dosedependentlystimulate growth of estrogen-dependent human breastcancer (MCF-7) tumors implanted in athymic nude mice. J. Nutr.; 131: 2957–2962.

11. Moore AB., Castro L., Yu L., Zheng X., Di X., Sifre MI.,Kissling GE., Newbold RR., Bortner CD., Dixon D(2007). Stimulatoryand inhibitory effects of genistein on human uterine leiomyoma cellproliferation are influenced by the concertration. Hum. Reprod.; 22: 2623–2631.

12. Kim YW., Hackett JC., Brueggemeier RW(2004). Synthesis andaromatase inhibitory activity of novel pyridine-containing isoflavones.J. Med. Chem.; 47: 4032–4040.

13. Mukherjee A., Dutta S., Sanyal U(2007). Evaluation of Dimethoxydop-NU as novel anti-tumor agent. Journal of Experimental Clinical Cancer Research; 26: 489–497.

14. Padmavathi V., Sudhakar RG., Padmaja A., Kondaiah P., Ali-Shazia(2009). Synthesis, antimicrobial and cytotoxic activities of1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles. EuropeanJournal of Medicinal Chemistry;44: 2106–2112.

15. Pain A., Samanta S., Dutta S(2003). Evaluation of Naphthalymustine,a nitrogen mustard derivative of naphthalimide as a rationallydesigned anticancer agent. Journal of Experimental Clinical Cancer Research;22: 411–418.

16. Robert W., Brueggemeier John CH., Edgar SD(2004). Aromatase inhibitors in the treatment of breast cancer. Endocrine Review;26(3): 331–345.

17. Roy MR., Guhathakurta S., Roychowdhury J(1981). Hematological changes in experimental tumors. Indian Journal of Medical Research; 74: 896–903.