CARDIOPROTECTIVE POTENTIAL OF HERBS AGAINST DOXORUBICIN INDUCED CARDIOTOXICITY: A REVIEW

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ABSTRACT:

Cancer is among the leading causes of death worldwide and its treatment includes radiotherapy, chemotherapy, surgery, hormonal therapy, and now a day's targeted therapy. Chemotherapy includes treatment of cancer by the use of certain drugs that causes damage or stress to the tumour cells. Doxorubicin is the commonly used drug to treat many types of cancer. Regardless of its great anti-tumour efficiency, it has several side effects. One of the major side effects is cardiotoxicity. Several mechanisms are proposed by which DOX induces cardiotoxicity. Out of them major one is oxidative stress. Antioxidants are the compounds that inhibit oxidation and can prevent damage to the cells caused by free radicals. Antioxidants can be derived from natural or artificial sources. Plants are rich in antioxidant and can be used as traditional and complementary medicine for the treatment of side effects associated with the doxorubicin.

Keywords: antioxidant, cancer, cardiotoxicity, chemotherapy, doxorubicin.

INTRODUCTION:

Cancer involves abnormal cell growth with the potential to invade or spread to other parts of the body (WHO and NCI, 2015). Cancer cure by various treatments includes chemotherapy, surgery, radiotherapy, and hormone therapy. Chemotherapy is the treatment of cancer with one or more anti-neoplastic drugs. The anti-neoplastic agents can be used along with other treatments, such as surgery or radiation therapy. Chemotherapeutic drugs act by killing cells that divide rapidly. Furthermore, chemotherapy can harms the cells that divide rapidly under normal conditions. The first anticancer agent (arsphen amine) was discovered in 1909 and used to treat syphilis (Gibaud et al, 2010). During the World War 1, mustard gas was discovered to be a potent agent for suppressor of hemato-poiesis (Krumbhar, 1919). It was reasoned that an agent damaged the white blood cells might have a similar effect on cancerous cells. Therefore, in December 1942, several patients with advanced lymphomas were treated. However, the first chemotherapy drug developed from

this line of research was Mustine. Since then, many other drugs have been developed to treat cancer (Gilman, 1963).

1 DOXORUBICIN (DOX):

DOX also called Adriamycin is produced by *Streptomyces peucetiusm* (Lomovskaya et al, 1999) is an anthracycline anticancer drug frequently used as a chemotherapeutic agent for various malignancies. Its chemical formula is $C_{27}H_{29}NO_{11}$ (Fig.1) having molecular weight 543.525 g/mol. It was approved for medical use in the United States and in 1974 included in the World Health Organization's List of Essential Medicines, as the most effective and safe medicines needed in a health system (WHO, 2015).

It is commonly used to treat some leukaemia and Hodgkin's lymphoma as well as cancers of bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others (The American Society of Health- System Pharmacists, 2016).

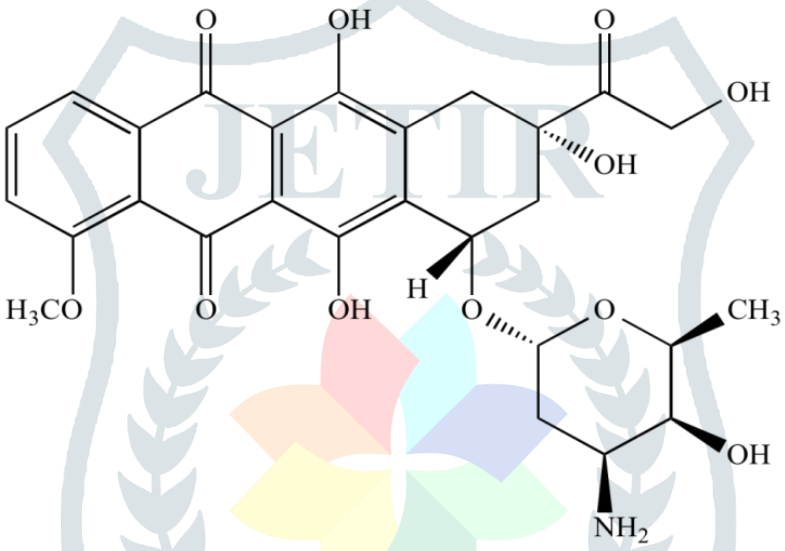

Figure 1 Skeletal formula of Doxorubicin

2 MECHAMISM OF ACTION:

Doxorubin interacts with DNA by intercalation (Fig.2); it inhibits the progression of topoisomerase II, which relaxes supercoils in DNA for transcription. It stop the process of replication by stabilizing the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication and may also increase free radical production, hence contributing to its cytotoxicity (Tacar et al, 2013; Fornari, 1994; Momparler et al, 1976). The planar aromatic chromophore portion of the molecule intercalates between the base pairs of the DNA, and the six membered daunosamine sugar sits in the minor groove and interacts with flanking base pairs just adjuscent to the intercalation site. And by intercalation, it can also induced histone eviction from transcriptionally active chromatin (Panget et al, 2013, 2015).

Finally, DNA repair, epigenome and transcriptomeare deregulated in doxorubicin-exposed cells (Tacar et al, 2013, Fornari et alm 1994; Momparler et al, 1976; Fredrick et al, 1990; Pigram et al, 1972).

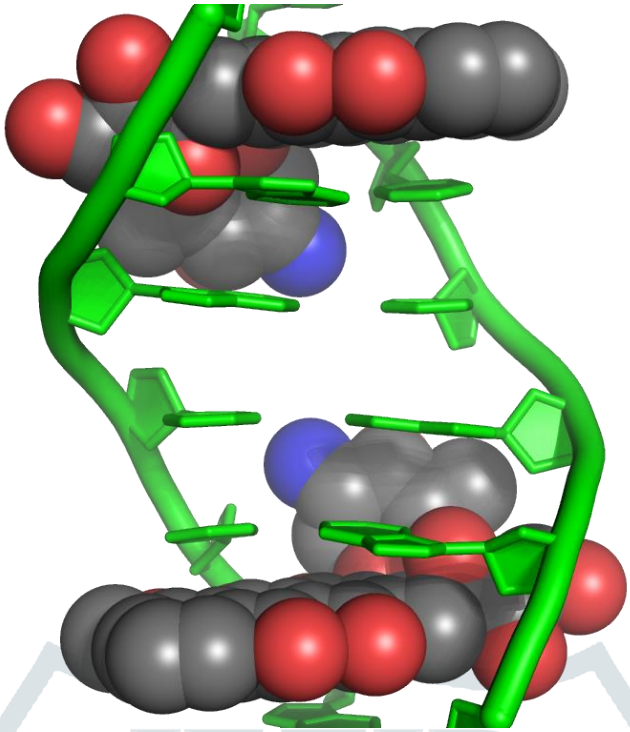

Figure 2 Two doxorubicin molecules intercalating DNA

3 SIDE EFFECTS:

DOX induced cardiopathy typically results in dilated cardiomyopathy, in which all four cardiac chambers get enlarged and results in both systolic and diastolic dysfunction. Eventually, it results into heart failure, which carries 50% mortality rate. There is no as such effective treatment against DOX induced cardiopathy till 2010 (Chaterjee et al, 2010). Various molecules such as dextrazoxane, beta blockers, leucovorin, erythropoietin amifostine, 2-mercaptoethane sulphonate sodium may be decrease the risk of doxorubicin's cardiotoxicity in certain cases without compromising the anticancer activity of DOX (Asselinet et al, 2016). Its prevention and management remains a great concern to both cardiologists and oncologists. And because of this use of doxorubicin is withdrawn and due to side effects and its red colour, doxorubicin has earned the nickname "red devil" or "red death".

Regardless of its great antitumor efficiency, its use in chemotherapy is limited due to its varied side effects including hair loss, bone marrow suppression, vomiting, rash, and inflammation of the mouth. The most prevalent and unavoidable side effect is cardiotoxicity, leading to congestive heart failure. Rate of cardiomyopathy is dependent on its cumulative dose, with an incidence about 4%, 8% and when its dose is 500–550 mg/m², 551–600 mg/m² and 600 mg/m² respectively.

4 DOX MECHANISM OF TOXICITY:

The mechanisms of cardiotoxicity are different from those of the mechanisms of its therapeutic effects of doxorubicin on tumour cells. It is possible that more than one mechanism is operative however, the proposed principal mechanisms of DOX cardiotoxicity are increased oxidative stress, as evident from increased levels of reactive oxygen species and lipid peroxidation, decreased levels of antioxidants and sulfhydryl groups, inhibition of nucleic acid and protein synthesis release of vasoactive amines, altered adrenergic function and decreased expression of cardiac-specific genes are other proposed mechanisms (Takemura, 2007).

4.1 OXIDATIVE STRESS

DOX appears to induce toxic damage to the cardiomyocyte's mitochondria. Several mitochondrial enzymes such as NADH dehydrogenase, cytochrome P-450 reductase and xanthine oxidase are involved in generating oxygen free radicals or ROS. It also increases superoxide formation by increasing endothelial nitric oxide synthase, which promotes intracellular hydrogen peroxide formation.

4.2 GENE EXPRESSION

The down regulation of α-actin, myosin light and heavy chains, troponin-I, and desmin proteins by DOX has been suggested as a potential mechanism of its cardiotoxicity. Decreased expression of the contractile proteins leads to myofibrillar loss and reduced myocardial contractile function. Down regulation of sarcoplasmic reticular ATPase can also cause abnormal myocardial diastolic function. It has been suggested that DOX can inactivate extracellular signal-regulated kinase (ERK) (Kim et al, 2003).

4.3 APOPTOSIS

There is evidence that DOX also induces apoptosis of cardiomyocytes. Formation of hydrogen peroxide and superoxide has been implicated in DOX-induced cardiomyocyte toxicity. These intracellular oxidants induce p53, and ultimately promote apoptosis of cardiomyocytes (Kim et al, 2003).

5 WHAT IS OXIDATIVE STRESS?

Oxidative stress (OS) is an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects trough neutralization by antioxidants. It reflexes an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. OS from oxidative metabolism causes base damage which is mostly indirect and caused by ROS generated, e.g. O_2^- (Superoxide radical), OH (Hydroxyl radical) and H_2O_2 (Hydrogen peroxide), as well as strand breaks in DNA (Chandrakala et al, 2015).

6 REACTIVE OXYGEN SPECIES

According to first theory of ROS induced aging, free radicals produced during the biological reactions leads to oxidative stress. This theory highlights a loss of the protective mechanisms that reduces the ability to oxidative challenges (Harman, 1956). The ROS are the natural by-product of the metabolism of oxygen and have important roles in cell signalling and homeostasis. Furthermore, free radicals are important contributor of pathological conditions including degenerative diseases. However during the environmental stress, the levels of ROS can increase dramatically, this will resulted in significant damage to cell structures (Devasagayam et al, 2004). The ROS could affect many cellular functions by oxidizing proteins, damaging nucleic acids and lipid per-oxidation, it is important to note that whether ROS will act as damaging, signalling or protective factors depends on the equilibrium between production and scavenging of ROS at the proper site of action (Brooker, 2011). Oxidative stress occurs in case of this critical balance due to depletion of antioxidants or accumulation of ROS in different body organs. Most ROS are byproducts of mitochondrial electron transport (Muller, 2000). Atomic oxygen has two unpaired electrons in separate orbits and this electron structure makes oxygen capable to radical formation. The

reduction of oxygen through the addition of electrons leads to formation of a number of ROS including: superoxide, hydrogen peroxide, hydroxyl radical, hydroxyl ion, and nitric oxide (www.google.com).

7 ANTIOXIDANTS:

Antioxidants sometimes called "free-radical scavengers" and are substances that inhibit oxidation and can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. Antioxidants can be naturally derived or artificial. These are the compounds that inhibit oxidation a reaction that can produce free radicals and may damage the cells of organism. Thiols and ascorbic acid are antioxidants that terminate these chain reactions. Plants and animals maintain a complex system of antioxidants such as glutathione and enzymes ex: catalase and superoxide dismutase to balance the OS. A number of defence mechanisms of body are evolved to provide a balance between production and removal of ROS in biological systems (www.google.com).

Superoxide dismutase (SOD) is catalyses the conversion of two superoxide anions into a molecule of hydrogen peroxide (H_2O_2) and oxygen (O_2).

$2O_2+2H+\rightarrow H_2O_2+O_2$

In the peroxisomes, the enzyme catalase converts H_2O_2 to water and oxygen, and completes the detoxification initiated by SOD.

$2H_2O_2\rightarrow 2H_2O+O_2$

There are also a number of non-enzymatic antioxidants molecules that play a role in detoxification in oxidative stress cases. Glutathione is the most important intracellular defence member against the reactive oxygen species. This tripeptide provides an exposed sulphydryl group, which serves as an abundant target for attack. The ratio of the Glutathione disulphide (GSSG) and the (Glutathione) GSH is a dynamic indicator of the oxidative stress of an organism (Hayyan et al, 2016). Glutathione peroxidase (GPx) is a group of enzymes containing selenium, which also catalyse the degradation of hydrogen peroxide, as well as organic peroxide to alcohols. The mechanism of free radical toxicity and subsequent detoxification by cellular enzymes is shown below (Fig.3).

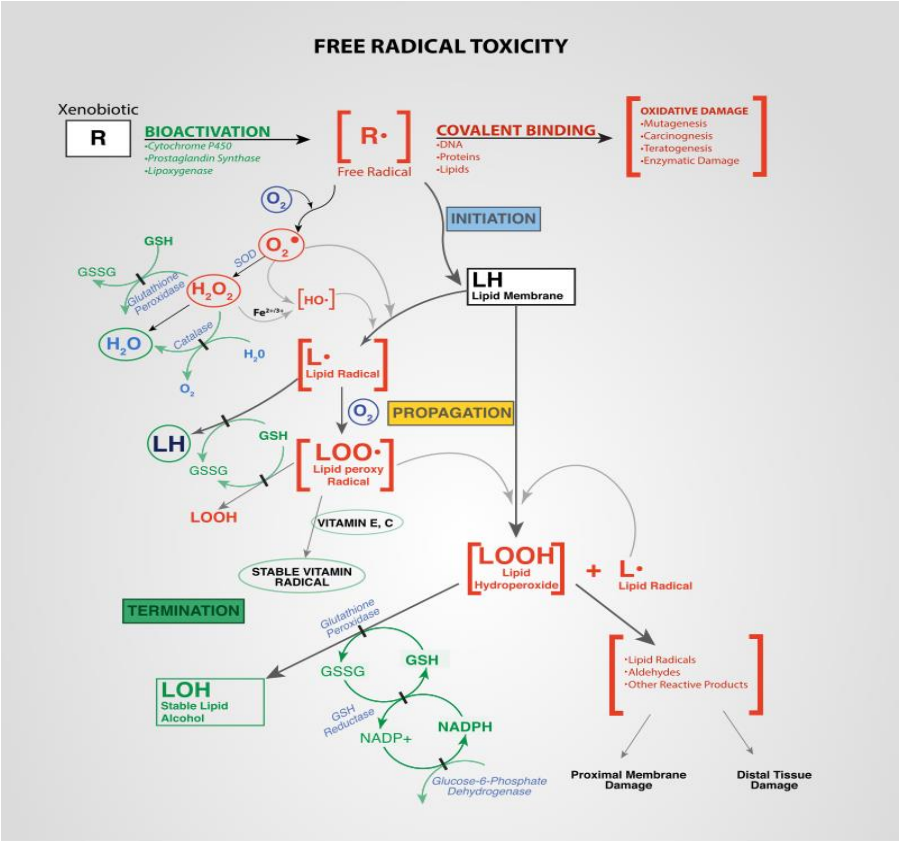

Figure 3 Free radical mechanism in tissue injury

Cardioprotective drugs against DOX-induced Cardiotoxicity:

As stated earlier to counteract DOX induced cardiotoxicity different drugs like melatonin, metformine, captopril, rosuvasatatin, omega 3, dexrazoxane, beta blockers, leucovorin, erythropoietin, amifostine, and 2-mercaptoethane sulphonate Na can be considered fesible candidates. These drugs are proved to have a protective effect against DOX- induced cardotoxicity (Ibrahim El- Sayed, 2018). However, these drugs got certain side effects also therefore there is an urgent need of herbal medicines to reduce the problem.

8 PLANTS USED TO REDUCE CARDIOTOXICITY:

Natural antioxidants are present in all parts of plants. These components belong to the class of phytochemicals such as phenols, carotenoids and flavonoids, which are capable of scavenging free radicals such as superoxide or lipid peroxides. The most current research on natural antioxidants has been focused on polyphenolic compounds such as flavonoids. Fruits, plant extracts and vegetables are rich sources of polyphenols, such as flavonoids and carotenoids, whose activities have been established in recent years. The anti- oxidative potential of plant based antioxidants resulted from the action of the cocktail of antioxidants present in plants (Dong-Ping et al, 2017).

Polyphenols and flavonoids are secondary metabolites of plants naturally occurring compounds characterized by the presence of phenol in their structure. Flavonoids have the general structure of a 15-carbon skeleton, which consist of two phenyl ring and a heterocyclic ring (Fig.4) (McNaught et al, 1997). Research on polyphenols and specially flavonoids and their antioxidant properties began after 1995. More than 8000 phenolic structure are known, and among them over 4000 flavonoids have been identified (Ververidiset et al, 2007).

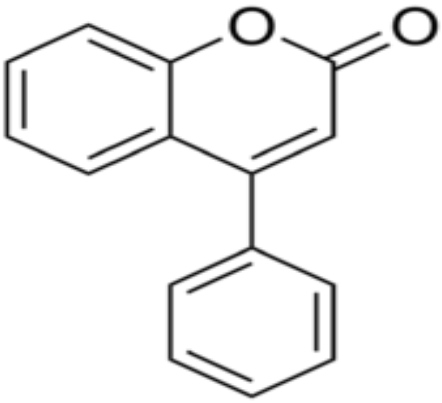

Figure 4 Structural formula of the neoflavonoid 4-phenyl coumarine (4-phenyl-1, 2-benzopyrone

Various plants have been studied to check the cardioprotective potential against DOX-induced cardiotoxicity.

8.1 *Solanum torvum* reduce DOX-induced cardiotoxicity in rats:

Solanum torvum Swartz commonly known as turkey berry belonging to family: Solanaceae. *S. torvum*, is found in tropical Africa, Asia and South America and is an important medicinal plant in tropical and subtropical countries is widely used like food and in folk medicine around the world. It is mainly used for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension. Pharmacological studies have also shown the ability of this plant to exhibit, cardiovascular, immunomodulatory, nephroprotective activity and anti-oxidant activity supporting its traditional uses (Jaiswal, 2012). A study on Wistar rats was conducted to check the protective effect of *Solanum torvum* against DOX induced cardiotoxicity. Cardiotoxicity was assessed by recording changes in ECG, heart rate and measuring the levels of cardiac marker enzymes- lactic acid dehydrogenase (LDH) and creatine phosphokinase (CK-MB) along with the antioxidant defence enzymes superoxide dismutase (SOD) and catalase (CAT) for heart tissue and histopathological changes were also measured. Results suggested that treatment with *S. torvum* (100 mg/kg and 300 mg/kg) significantly reversed the changes in Electrocardiography (ECG); decreased the levels of CK-MB and LDH; and increased the antioxidant defence enzyme levels of SOD and CAT as well as *S. torvum* treated rats showed a lesser degree of cellular infiltration in histopathological studies (Kamble et al, 2009).

Nephrotoxicity is also one of the major side effects of antracycline antobiotics. A study was conducted to determine the protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats using biochemical and histopathological approaches. Treatment with *S. torvum* fruits (100mg/kg and 300mg/kg) significantly decreased the levels of creatinine and Blood Urea Nitrogen (BUN) and significantly increased the anti- oxidant defence enzyme levels of Superoxide Dismutase (SOD) and catalase (CAT). Histopathological change showed that DOX caused significant structural damages to kidney like tubular necrosis, renal lesions and glomerular congestion which were reversed with *S. torvum* and hence the results that *S. torvum* has the potential in preventing the nephrotoxicity induced by doxorubicin (Mohan et al, 2010).

8.2 Cardioprotective effect of Root extract of *Glycyrrhiza uralensis* on DOX induced cardiotoxicity in mice:

Radix *Glycyrrhizae*is considered to be the ‘‘King of Herbs’’ (Kim et al, 2006) and possesses various pharmaceutical anticarcinogenic, anti-inflammatory and antiulcer

properties (Sheela et al, 2006; Kim et al, 2008; Ji et al, 2007) is used in foods, beverages, candies, tobacco, and dietary supplements s in the United States, Europe, the Middle East, and Russia (Lin et al, 2009). Glycyrrhizin, the main water-soluble constituent of licorice, was used as antidote for saponin, alkaloid urethan, benzene, carbon tetrachloride (CCl4)-induced toxicity, (Agarwal et al, 1991) and exerted antigenotoxic and hepatoprotective effects (Isbrucker, 2006).

Glycyrrhizic acid was also reported to protect against aflatoxin-induced oxidative stress with its capacity to inhibit the metabolic activation of hepatotoxin (Chan et al, 2003). *Glycyrrhizae inflata* attenuates the toxicity of cisplatin without compromising its antitumor effects (Lee et al, 2007). *Glycyrrhiza uralensis* is the most commonly used in China. Very recently, researchers reported that hexane andethanol extract of *Glycyrrhiza uralensis* suppresses DOX-induced apoptosis in H_9C_2 cardiac cell lines (Choi et al, 2008).Treatment with *Glycyrrhiza uralensis* extract (GUE) significantly protected the mice from DOX-induced cardiotoxicity, indicated by decreased levels of serum LDH and CK-MB, improved heart morphology and increased Glutathione peroxidase (GSH-PX) activity and Glutathione (GSH) level without compromising thetumour-inhibitory effect of DOX (Ling et al, 2011).

8.3 Cardioprotective effects of ethanolic extract of *Phyllanthus urinaria* L. on DOX-Induced Cardiotoxicity:

In Ayurveda medicine, for more than 2000 years, *Phyllanthus* spp. (Euphorbiaceae) has been usedas traditional herbs, also in Thai folk medicine for the treatment of many diseases, such as diarrhoea, hepatitis, diabetes, abdominal pain, and kidney disease. Some works have been reported for its antioxidative potential. For example, *Phyllanthus amarus* reduced oxidative damage from radiation and protect against hepatotoxicity induced by carbon tetrachloride (CCl4) (Tasaduq et al, 2003). *P. emblica* protected rat hearts from OS induced by ischemic-reperfusion injury (Rajak et al, 2004). *P. urinaria* L. is another medicinal plant that is used in the treatment of several diseases. Several potential antioxidant compounds e.g., polyphenols, lignans, flavonoids, gallic acid, ellagic acid, etc. have been isolated from this plant (Chang et al, 2003; Zhang et al, 2000).

Phyllanthus urinaria (PU) ethanolic extract treatments (1 or 10 mg/ml) dose dependently causes rightward DOX IC50 shifts of 2.8- and 8.5-fold, respectively while treatments with ascorbic acid and N-Acetylcysteine (NAC)increased DOX IC50 by 3.3- and 4.2-fold, respectively. Additionally, all antioxidants completely inhibited cellular lipid peroxidation and caspase-3 activation induced by DOX (1mM). Antioxidants also modulated endogenous antioxidant defence such as total glutathione (tGSH), catalase and superoxide dismutase (SOD) activity. As well as, the nuclear factor kB (NFkB) transcription factor assay demonstrated that all antioxidants significantly inhibited DOX-induced NFkB activation. These results suggest that PU protect against DOX-induced cardiotoxicity and this plant may serve as an alternative source of antioxidants for prevention of DOX cardiotoxicity.

8.4 Fatty Acids Ameliorate Doxorubicin-Induced Intracellular Ca2+ Increase and Apoptosis in Rat Cardiomyocytes:

Magnolia officinalis used as a traditional medicine in Chinese, Korean, and Japanese for the treatment of various diseases has been known to treat cold-stroke, cold damage, headache, and blood impediment (Fujita et al, 1973). Bioactive constituents Honokiol and Magnolol are present in the stem barks of *M. obovata* and *M. officinalis* (Zhai et al, 2005).

To inhibit angiogenesis in vitro and tumour growth in vivo as well as lipid peroxidation in rat heart mitochondria, and arrhythmia in coronary ligated rats (Bai et al, 2003; Tsai et al, 1996). Magnolol is also reported to reduce myocardial ischemia in rat via the inhibition of neutrophils migration (Lee et al, 2001).

DOX-induced cardiotoxicities are known to be caused mainly by ROS generation, resulting in elevation of intracellular Ca2+ concentration through release from sarcoplasmic reticulum. Researchers investigated the effects of *Magnolia* seed extract (MagS) on the DOX- induced cardiotoxicity, and found that MagS significantly reduces DOX-induced increase in intracellular Ca2+ concentration generation of ROS, and apoptosis in rat cardiomyocytes. The bioactive compounds in MagS are linoleic acid, oleic acid, and palmitic acid and were able to inhibit the DOX-induced increase in Ca2+, ROS generation, and apoptosis with a similar potency. Treatment of the MagS (2mg/kg/d, intraperitoneally) substantially attenuated the DOX -induced cardiac damages including the loss of body weight. These results suggest that fatty acids in MagS and other seeds may ameliorate cardiotoxicity of the DOX (Kwang-Hyun et al, 2008).

8.5 Total Flavonoids from *Clinopodium chinense* (Benth) O. Ktze (TFCC) supress all the changes associated with DOX:

Clinopodium chinense (Benth) is a traditional Chinese herbal medicine which belongs to the family Labiatae. Various reports had shown that the stem and leave of *C. chinense* (Benth.) O. Ktzehas various pharmacological effects, such as anti-inflammatory and immunity (Li, 1989, 1983), lowering blood glucose (Tian et al, 2008), and antitumor and antiradiation (Chen et al, 2012; Dzhambazov et al, 2002). The major components in the *C. chinense* (Benth) include flavonoids, triterpenoid, saponins, and volatile oil (Chen et al, 2012). Total flavonoid from *C. chinense* (Benth) (TFCC) plays a major role in the treatment of cardiovascular disease (CVD).

TFCC pre-treatment in rats and cell lines suppressed all of the adverse effects of doxorubicin. Signal transduction studies shows that TFCC suppressed DOX-induced overexpression of p53 and phosphorylation of c-Jun N-terminal kinase (JNK), p38, and dextracellular signal- regulated kinases (ERK). These findings proved the potential clinical application of TFCC in preventing DOX-induced cardiac Oxidative stress (Chen et al, 2015).

8.6 Ephedra nebrodensis ameliorate Doxorubicin-Induced Cardiotoxicity in Rats:

Ephedra nebrodensis contain ephedrine medicinally-active alkaloids and they are widely used in preparations for the treatment of asthma and catarrh (Bown, 1995). The species is the richest source of ephedrine in India; the stems contain over 2.5% total alkaloids, of which about 75% is ephedrine having diaphoretic, diuretic, febrifuge, hypertensive, nervine, tonic, vasoconstrictor and vasodilator properties (Chopra, 1986). These plants also have antiviral effects, particularly against influenza and are used internally in the treatment of asthma, hay fever and allergic complaints (Bown, 1995; Yeung, 1985).

Treatment with *Ephedra nebrodensis* (100 mg/kg and 200 mg/kg) significantly decreased the levels of lipoperoxidase (LPO) and cardiac marker enzymes and increased the levels of other antioxidant defence enzymes such as GSH and SOD along with reversal changes in ECG and prevented the decrease in heart weight in DOX-treatedanimals. The results suggest that *Ephedra nebrodensis* has the potential in preventing the cardiotoxicity induced by Doxorubicin (Shah et al, 2009).

8.7 Grape Seed and Skin Extract Protects against Acute Chemotherapy Toxicity induced by Doxorubicin in rat heart:

Grape seed and skin extract (GSE) is an excellent scavenger of hydroxyl, superoxide and other free radicals. It also protects against lipid peroxidation in cell membranes and DNA damage caused by ROS generation (Leonard et al, 2003). Furthermore, the use of GSE as a cardioprotective agent is emphasized because of the low toxicity and direct anticancer activity of its polyphenol content (Agarwal et al, 2004; Jang et al, 1997; Rezk et al, 2006). The combination of resveratrol, a polyphenol mainly present in GSE, and doxorubicin has even been reported to have an additive benefit against uterine and ovarian cancer cells (Rezk et al, 2006).

Pre-treatment with grape seed and skin extract (GSE), commonly used as an antioxidant agent, may alleviate DOX induced cardiotoxicity. When rats were treated with GSE (500 mg/kg bw) by intraperitoneal injection it was observed that GSE counteracted DOX-induced disturbances of hemodynamic parameters, alleviated oxidative stress as assessed by normalized iron and Ca2+levels and increased SOD activity.

8.8 Saffron extracts alleviate cardiomyocytes injury induced by doxorubicin and ischemia-reperfusion in vitro:

Saffron is a rich source of flavonoids .Saffron extract (SAF), mainly consisting of safranal and corcins, exerts a protective effect against DOX oxidative cytotoxicity in isolated rabbit hearts. In another they have investigated whether SAF exerts cardioprotection against combined is chemiareperfusion (I/R) and DOX toxicity in H_9C_2 cardiomyocytes. The I/R and DOX significantly decreased cardiomyocytes viability, inhibited reperfusion injury salvage kinase cardioprotective pathway, reduced contractile proteins, increased caspase-3 expression and induced loss of mitochondrial membrane potential. These effects were significantly inhibited by treatment with SAF (10 lg/mL) at reperfusion. SAF restored contractile proteins expression, inhibited mitochondrial permeability transition pore and decreased caspase-3 activity. Findings indicate that SAF treatment exerted cardioprotection against I/R and DOX toxicity by reducing oxidative stress and offers a potential novel antioxidant therapeutic strategy to counteract I/R and DOX cardiotoxicity.

8.9 Cardioprotective effects of Lycopene and Tomato extract:

Lycopene is the most prominent carotenoid in tomatoes (*Lycopersicone sculentum*), and is relatively resistant to heat. Carotenoids exert antioxidant activity, and among these lycopene exhibits the highest, almost double as that of b-carotene (DiMascio et al., 1989). DOX toxicity induced by a single intraperitoneal injection (15 mg/kg), shows elevated Creatine Phosphokinase-MB (CPKMB) and histopathological observations showed cardiac cell injury. Tomato extract (1.2 and 2.4 g/kg, i.p.) and lycopene (1.7 and 3.5 mg/kg, i.p.), prevented the rise in serum CPKMB and ameliorated cardiac cell injury, suggesting that tomato extract and lycopene inhibit doxorubicin-induced cardiotoxicity and may serve as a combination chemotherapeutic agent with DOX to limit its cardiotoxic effects (Gholamreza et al, 2005).

8.10 Bark of *Terminalia arjuna* has cardioprotective potential against Doxorubicin-induced cardiotoxicity:

The bark of *Terminalia arjuna*, a deciduous tree of Combretaceae family, has been used as a potential cardioprotective agent since vedic period, has been widely used since Vedic period for the treatment of various heart diseases. It is known as "Hridya", as it possesses heart strengthening and cardiotonic properties. *Terminalia arjuna* bark powder mixed with milk for the relief of chest pain caused by heart was first used (Vagabhatta, 1963).

There was a significant decrease in myocardial superoxide dismutase (38.94%) and reduced glutathione (23.84%) in animals treated with 20mg/kg DOX. A marked increase in serum creatine kinase-MB (CKMB) activity (48.11%) as well as increase in extent of lipid peroxidation was also reported. Co-treatment of butanolic fraction of *Terminalia arjuna* (TA) bark and DOX resulted in an increase in the cardiac antioxidant enzymes, decrease in serum CKMB levels and reduction in lipid peroxidation as compared to DOX-treated animals. Electron microscopic studies in DOX-treated animal's revealed mitochondrial swelling, Z-band disarray, focal dilatation of smooth endoplasmic reticulum (SER) and lipid inclusions, whereas the concurrent administration of TA butanolic fraction led to a lesser degree of DOX-induced histological alterations, suggesting that butanolic fraction of *Terminalia arjuna* bark has protective effects against DOX-induced cardiotoxicity and may have potential as a cardioprotective agent.

Study conducted in cell lines and on mice showed that (1–100 µg/ml) aqueous extract of *Terminalia arjuna*bark (TAAqE) reduced OS and preserved mitochondria and cell growth of H9c2 cells against DOX treatment (Bishopa and Liua, 2017). TAAqE (in drinking water) attenuated the decreased Left Ventricular function and altered myocardial structure caused by DOX treatment. TAAqE exerts a protective action against cardiotoxicity caused by DOX in part via suppression of OS suggesting that TAAqE is a promising cardiotonic in adjuvant cancer chemotherapy.

8.11 Cardioprotective activity of *Stachys schimperi* Vatke:

Genus *Stachysis* is one of the largest genera of the family Lamiaceae. Plants belonging to this genus have been used in folk medicine for centuries to treat genital tumours, sclerosis of the spleen, cough, inflammatory diseases and ulcers (Hartwell, 1982). Methanolic extract of *Stachys schimperi* showed moderate protection against DOX-induced alteration in cardiac oxidative stress markers; GSH and Malondialdehyde (MDA), and cardiac serum markers; CK-MB and Lactate dehydrogenase (LDH) activities in rats. Isoscutellarein 7-O-[200-O-(6000-acetyl)-b-D-allopyranosyl]-b-D-glucopyranoside was isolated and identified as a major constituent for the first time from this *Stachys* species.

8.12 p-coumaric acid protects rat's heart against DOX-induced OS:

p-coumaric (PC) acid (100mg/kg), a member of phenolic acids, widely distributed in plants and form a part of human diet (Scalbert et al, 2000) significantly reduced DOX-induced high serum levels of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK). Pretreatment with PC also ameliorated the cardiac content of glutathione (GSH), and superoxide dismutase (SOD) and catalase (CAT) activities, accumulation of cardiac content of MDA significantly decrease compared to DOX-receiving rats. The result indicates that PC protects rat's hearts against DOX-induced oxidative stress in the heart.

8.13 Effect of *Callistemon lanceolatus* leaves on DOX-induced cardiomyopathy in rats:

Callistemon lanceolatus is commonly known as bottle brush because of its crimson red colored spikes. It belongs to family Myrtaceae and is frequently cultivated throughout India as ornamental plants (Sharma et al, 2006). It is indigenous to Australia. The Lambadi tribal of north Telangana districts of Andhra Pradesh use this plant for the treatment of infectious diseases pain and gastrointestinal disorders. Aqueous extracts of the leaves and flowers have antimicrobial activity. The plant extracts inhibit urd bean leaf crinkle virus in vitro and also shows cholinesterase activity (Chowdhary and Saha, 1985; Gupta and Gupta, 1997). The essential oils from leaves possess antimicrobial, fungitoxic, antinociceptive and anti–inflammatory activities and was also reported for its hepatoprotective effect (Jain et al, 2007).

Pre-treatment of ethanol extract of *Callistemon lanceolatus* leaves (100 and 200 mg/kg) on rat shows cardioprotective effect against doxorubicin-induced cardiomyopathy. 200 mg/kg extract significantly reduced the elevated levels of the serum enzymes and restores the ECG and blood pressure to normal, also significantly increased the tissue anti-oxidant levels, while decreased the malondialdehyde level.

CONCLUSION:

Doxorubicin is an effective anticancer drug that is used to treat various type of cancers such as leukaemia, Hodgkin's lymphoma as well as cancers of bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Cardiotoxicity is unavoidable and major side effect of this drug due to which patients die because of dilated cardiomyopathy. Oxidative stress or generation of ROS is one of the well understood mechanisms of its toxicity. Various other drugs have been designed to overcome this however; they also have serious side effects. Naturally derived antioxidants can be used to fight against the Doxorubicin-induced cardiotoxicity. Plants being a rich source of antioxidants and studies on their antioxidative and cardioprotective potential have proved that they can be used as adjuvants along with anthracycline mediated cancer chemotherapy. Plants such as *Solanum torvum, Glycyrrhiza uralensis, Phyllanthus urinaria* L., *Clinopodium chinense, Ephedra nebrodensis,* Saffron, Grape Seed, *Lycopersicone sculentum, Callistemon lanceolatus, Stachys schimperi* Vatke,*Terminalia arjuna,* etc. have been already used in Ayurveda for many years and research using animal model and on cell lines has shown that they got antioxidant as well as cardioprotective potential ameliorating the alteration associated with DOX induced cardiotoxicity.

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REFERENCES:

[1] Agarwal R, Wang, Z, and Mukhtar, H. 1991. Inhibition of mouse skin tumor-initiating activity of DMBA by chronic oral feeding of glycyrrhizin in drinking water. Nutr Cancer, 15(3-4): 187-193.

[2] Agarwal, BB, Bhardwaj, A, Agarwal, RS, Seeram, NP, Shishodia, S, and Takada, Y. 2004. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Research, 24: 2783–2840.

[3] Asselin, BL, Devidas, M, Chen L. 2016. Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed t-cell acute lymphoblastic leukemia or advanced stage lymphoblastic non-hodgkin lymphoma: a report of the children's oncology group randomized trial pediatric oncology group 9404. Journal of Clinical Oncology, 34(8): 854–862.

[4] Bai, X, Cerimele, F, Ushio-Fukai, M, Waqas, M, Campbell, PM, Govindarajan, B, Der, CJ, Battle, T, Frank, DA, Ye, K, Murad, E, Dubiel, W, Soff, G, Arbiser, JL. 2003. Honokiol, a small molecular weight natural product, inhibits angiogenesis in vitro and tumor growth in vivo. J. Biol. Chem. 278: 35501-35507.

[5] Bishop, S, Shi, JL. 2017. Cardioprotective action of the aqueous extract of *Terminaliaarjuna* bark against toxicity induced by doxorubicin. Phytomedicine, 36: 210-216.

[6] Bown, D. 1995. Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London.

[7] Brooker, RJ. 2011. Genetics: analysis and principles (4th ed). McGraw-Hill Science.

[8] Chan, HT, Chan, C, HoJ, W. 2003. Inhibition of glycyrrhizic acid on aflatoxin B1-induced cytotoxicity in hepatoma cells. Toxicology, 188(2-3): 211-217.

[9] Chandra, K, Syed, SA, Abid, M, Rajpoot, S, Khan, NA. 2015. Protection against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costusspeciosus Rhizome Extract. International Journal of Pharmacognosy and Phytochemical Research, 7 (2): 383–389.

[10] Chang, CC, Lien, YC, Liu, KC, Lee, SS. 2003. Lignans from *Phyllanthus urinaria*. Phytochemistry, 63: 825-833.

[11] Chatterjee, K, Jianqing, Z, Norman, H, Joel, SK. 2010. Doxorubicin Cardiomyopathy. Cardiology, 115(2): 155-162.

[12] Chen, K, Wu, FH, Yan, HS, Qu, W and Liang, JY. 2012. Advances in studies on chemical constituents in Clinopodium and their pharmacological activities. Strait Pharmaceutical Journal, 24(7): 6-10.

[13] Choi, HJ, Seon, MR, Lim, SS, Kim, JS, Chun, HS, Park, JH. 2008. Hexane/ ethanol extract of Glycyrrhiza uralensis licorice suppresses doxorubicin-induced apoptosis in H9c2 rat cardiac myoblasts. Exp Biol Med (Maywood), 233(12):1554-1560.

[14] Chopra, RN, Nayar, SL, Chopra, IC. 1986. Glossary of Indian Medicinal Plants, New Delhi, India. Council of Scientific and Industrial Research.

[15] Chowdhary, AK, Saha, NK. 1985. Inhibition of urd bean leaf crinkle virus by different plant extracts. Indian Phytopathol, 38:566-68.

[16] Devasagayam, TP, Tilak, JC, Boloor, KK, Sane, KS, Ghaskadbi, SS, Lele, RD. 2004. Free radicals and antioxidants in human health: current status and future prospects. The Journal of the Association of Physicians of India, 52:794-804.

[17] Dong-Ping, X , Ya Li , Xiao Meng, Tong Zhou, Yue Zhou, Jie Zheng Jiao-Jiao Zhan, Hua-Bin Li. 2017. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. P International journal of molecular sciences, 18(1): 96.

[18] Dzhambazov, B, Daskalova, S, Monteva, A and Popov, N. 2002. In vitro screening for antitumour activity of Clinopodiumvulgare L. (Lamiaceae) extracts. Biological and Pharmaceutical Bulletin, 25(4): 499-504.

[19] Fornari, FA, Randolph, JK, Yalowich, JC, Ritke, MK, Gewirtz, DA. 1994. Interference by doxorubicin with DNA unwinding in MCF-7 breast tumor cells. Mol Pharmacol, 45(4): 649-656.

[20] Frederick, CA, Williams, LD, Ughetto, G. 1990. Structural comparison of anticancer drug-DNA complexes: adriamycin and daunomycin. Biochemistry, 29(10): 2538-2549.

[21] Fujita, M, Itokawa, H, Sashida, Y. 1973. Studies of the compounds of Magnolia obovata Thunb. III. Occurrence of magnolol and honokiol in M. obovata and other allied plants. Yakugaku Zasshi, 93: 429-434.

[22] Gholamreza, K, Mohammad, R, Azadeh, A. 2005. Effects of Lycopene and Tomato Extract against Doxorubicin-Induced Cardiotoxicity. Iranian Journal of Pharmaceutical Sciences Spring, 1(2): 85-90.

[23] Gibaud, Stéphane; Jaouen, Gerard. 2010. Arsenic- based drugs: from Fowler's solution to modern anticancer chemotherapy. Topics in Organometallic Chemistry, 32: 1–20.

[24] Gilman, A. 1963. The initial clinical trial of nitrogen mustard. Am. J. Surg., 105(5): 574-578.

[25] www.google.com

[26] Gupta, A and Gupta, R. 1997. A survey of plants for anticholinesterase activity. Phytochemistry, 46: 827-31.

[27] Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology, 11(3): 298-300.

[28] Hartwell, JL. 1982. Plants used against cancer. A survey. Massachusetts: Quarterman Publications Inc.

[29] Hayyan, M, Hashim, MA, Nashef, IM. 2016. Superoxide Ion: Generation and Chemical Implications. Chem. Rev., 116(5): 3029-3085.

[30] Ibrahim El-Sayed 2018. Different Drugs are tried to counteract the Cardio toxicity of Doxorubicin and their Suggested Mechanisms: A Review. J Med Oncol., 1(3): 1-8.

[31] Isbrucker, RA, Burdock, GA. 2006. Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol; 46(3):167-192.

[32] Jain, AK, Dubey, SK, Sikarwar, MS, Jain, SK. 2007.Hepatoprotective activity of methanolic extract of leaves of *Callistemon lanceolatus*. Internat J Plant Sci.; 2:185- 186.

[33] Jaiswal, BS. 2012. *Solanumtorvum*: a review of its traditional uses, phytochemistry and pharmacology. Int J Pharm Bio Sci., 3(4): 104-111.

[34] Jang, M, Cai, L, Udeani, GO, Slowing, KV, Thomas, CF, Beecher, CW. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science, 275: 218–220.

[35] Ji, XL, Jiang, YX, Ren, BB, Liu, K, Liu, G. 2007. Effect of bismuth glycyrrhizate on experimental gastric ulcers and its mechanisms. Zhongguo Zhong Yao ZaZhi, 32(14): 1429-1432.

[37] Kamble, S, Mohan, M, Kasture, S. 2009. Protective Effect of *Solanum torvum* on Doxorubicin-Induced Cardiactoxicity in Rats Pharmacologyonline, 2:1192-1204.

[38] Kim, DC, Choi, SY, Kim, SH. 2006. Isoliquiritigenin selectively inhibits H (2) histamine receptor signaling. Mol Pharmacol., 70(2): 493-500.

[39] Kim, JY, Park, SJ, Yun, KJ, Cho, YW, Park, HJ, Lee, KT. 2008. Isoliquiritigenin isolated from the roots of Glycyrrhiza uralensis inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. Eur J Pharmacol., 584(1): 175-18.

[40] Kim, Y, Ma, AG, Kitta, K, Fitch, SN, Ikeda, T, Ihara, Y, Simon, AR, Evans, T, Suzuki 2003. Anthracycline-induced suppression of GATA-4 transcription factor: implication in the regulation of cardiac myocyte apoptosis. J. Mol. Pharmacol., 63(2): 368-77.

[41] Krumbhaar, EB. 1919. Tole of the blood and the bone marrow in certain forms of gas poisoning. JAMA., 72:39-41.

[42] Kwang-Hyun Park, Seon-Young Kim, Rukhsana Gul, Byung-Ju Kim, Kyu Yun Jang, Hun-Taeg Chung, and Dong-Hwan Sohn. 2008. Fatty Acids Ameliorate Doxorubicin-Induced Intracellular Ca2 Increase and Apoptosis in Rat Cardiomyocytes. Biol. Pharm. Bull., 31(5): 809-815.

[43] Lee, CK, Park, KK, Lim, SS, Park, JH, Chung, WY. 2007. Effects of the licorice extract against tumor growth and cisplatin-induced toxicity in a mouse xenograft model of colon cancer. Biol Pharm Bull., 30(11): 2191-2195.

[44] Lee, YM, Hsiao, G, Chen, HR, Chen, YC, Sheu, JR, Yen, MH. 2001. Magnolol reduces myocardial ischemia/ reperfusion injury via neutrophil inhibition in rats. Eur. J. Pharmacol., 422: 159-167.

[45] Leonard, SS, Xia, C, Jiang, BH, Stinefelt, B, Klandorf, H, Harris, GK. 2003. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochemical and Biophysical Research Communications, 309: 1017-1026.

[46] Li, G. 1989. Hemostatic and anti-inflammatory effect of Clinopodium polycephalum, Journal of Biology, 3(1): 138.

[47] Li, G. 1993. Inhibitative effect of total saponins of Clinopodiumpolycephalum on immune function, Chinese Traditional and Herbal Drugs, 3(3): 138.

[48] Lin, SP, Tsai, SY, Hou, YC, Chao, PD. 2009. Glycyrrhizin and licorice significantly affect the pharmacokinetics of methotrexate in rats. J Agric Food Chem., 57(5): 1854-1859.

[49] Ling, Z, Yinxin, Y, Lingyan, Y, Yi, W, Li, L and Xiaohui, F. 2011. Cardioprotective Effects of Glycyrrhizauralensis Extract Against DoxorubicinInduced Toxicity. International journal of Toxicology, 30(2): 181-189.

[50] Lomovskaya, N, Otten, SL, Doi-Katayama, Y. 1999. Doxorubicin overproduction in Streptomyces peucetius: cloning and characterization of the dnrUketoreductase and dnrV genes and the doxA cytochrome P-450 hydroxylase gene. J. Bacteriol., 181(1): 305–318.

[51] McNaught, AD, Wilkinson, A, 1997. IUPAC Compendium of Chemical Terminology (2nd ed), Oxford: Blackwell Scientific.

[52] Minotti, G, Menna, P, Salvatorelli, E, Cairo, G, Gianni, L. 2004. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev., 56(2): 185-229.

[53] Mohan, M, Kamble, S, Gadhi, P, Kasture, S. 2010. Protective effect of *Solanum torvum* on doxorubicin-induced nephrotoxicity in rats. Food and Chemical Toxicology, 48(1): 436-440.

[54] Momparler, RL, Karon, M, Siegel, SE, Avila, F. 1976. Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells. Cancer Res., 36(8): 2891–2855.

[55] Muller, F. 2000. The nature and mechanism of superoxide production by the electron transport chain: Its relevance to aging. Journal of the American Aging Association, 23 (4): 227-253.

[56] National Cancer Institute, 2018.

[57] Pang, B, de Jong, J, Qiao, X, Wessels, LF, Neefjes, J. 2015. Chemical profiling of the genome with anti-cancer drugs defines target specificities. Nature Chemical Biology, 11(7): 472-480.

[58] Pang, B, Qiao, X, Janssen, L, Velds, A, Groothuis, T, Kerkhoven, R, Nieuwland, M, Ovaa, H, Rottenberg, S, van Tellingen, O, Janssen, J, Huijgens, P, Zwart, W, Neefjes, J. 2013. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nature Communications, 4(5): 1908.

[59] Pigram, WJ, Fuller, W, Hamilton, LD. 1972. Stereochemistry of intercalation: interaction of daunomycin with DNA. Nature New Biology, 235(53): 17-19.

[60] Rajak, S, Banerjee, SK, Sood, S, Dinda, AK, Gupta, YK, Gupta, SK, Maulik, SK. 2004. Emblicaofficinalis causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury in rats. Phytother. Res., 18: 54-60.

[61] Rezk, YA, Balulad, SS, Keller, RS and Bennett, JA. 2006. Use of resveratrol to improve the effectiveness of cisplatin and doxorubicin: study in human gynecologic cancer cell lines and in rodent heart. American Journal of Obstetrics and Gynecology, 194: 23-26.

[62] Scalbert A, Williamson G. Dietary intake and bioavilability of polyphenols (2000) J Nutr;130(8 Suppl):2073S–85.

[63] Shah, S, Mohan, MM, Kasture, S, Sanna, C and Maxia, A. 2009. Protective Effect of Ephedra nebrodensis on Doxorubicin-Induced Cardiotoxicity in Rats.Iranian Journal of Pharmacology and Therapeutics, 8(2): 61-66.

[64] Sharma, RK, Kotoky, R, Bhattacharyya, PR. 2006. Volatile oil from the leaves of *C. lanceolatus* D. C. grown in north-eastern India. Flavour Fragr J., 21: 239-240.

[65] Sheela, ML, Ramakrishna, MK, Salimath, BP. 2006. Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by Glycyrrhiza glabra. Int Immuno. pharmacol, 6(3): 494-498.

[66] Tacar, O, Sriamornsak, P, Dass, CR. 2013. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. The Journal of Pharmacy and Pharmacology, 65(2): 157-170.

[67] Takemura, G, Fujiwara, Prog H. 2007. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. Cardiovasc Dis., 49(5): 330-352.

[68] Tasaduq, SA, Singh, K, Sethi, S, Sharma, SC, Bedi, KL, Singh, J, Jaggi, BS, Johri, RK. 2003. Hepatocurative and antioxidant profile of HP-1, a poly herbal phytomedicine Hum. Exp. Toxicol., 22: 639-645.

[69] The American Society of Health-System Pharmacists, 2017.

[70] Tian, DN, Wu, FH, Ma, SC, Li, D and Dai, Y. 2008. Studies on anti-hyperglycemic effect and its mechanism of Clinopodiumchinense. China Journal of Chinese Materia Medica, 33(11): 1313-1316.

[71] Tsai, SK, Huang, SS, Hong, CY. (1996). Myocardial protective effect of honokiol: an active component in Magnolia officinalis. Planta Med., 62: 503-506.

[72] Vagabhatta. 1963. Commentaries by Pandit Lalchandra Vaidya. In: Astang Hridyam, 1st ed. Motilal Banarasi Das, Varanasi, India.

[73] Ververidis, F, Trantas, E, Douglas, C, Vollmer, G, Kretzschmar, G, Panopoulos, N. 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. Biotechnology Journal, 2(10): 1214-1234.

[74] Wang, X, Wang, Y, Geng, Y, Li, F, Zheng, C. 2004. Isolation and purification of honokiol and magnolol from cortex Magnoliaeofficinalis by high-speed counter-current chromatography. Journal of Chromatography, 1036(2): 171-175.

[75] World Health Organization, 2016 and 2018.

[76] Yeung, HC. 1985. Handbook of Chinese Herbs and Formulas. Inst of Chinese Med.

[77] Zhai, H, Nakade, K, Oda, M, Mitsumoto, Y, Akagi, M, Sakurai, J, Fukuyama, Y. 2005. Honokiol-induced neurite outgrowth promotion depends on activation of extracellular signal-regulated kinases (ERK1/2). European Journal of Pharmacology, 516(2): 112-117.

[78] Zhang, LZ, Guo, YJ, Tu, GZ, Guo, WB, Miao, F, Zhongguo 2000. Studies on chemical constituents of Phyllanthusurinaria L Zhong Yao ZaZhi, 25: 615-617.