Review article on the screening of Cardioprotective and Anti-oxidant activity.

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Abstract:

Cardiovascular diseases are the most widespread and topmost lives and death claim global disease. Studies show that, this chronic illness dose not only influence on people above 60's, but also have an adverse effect for people from the age of 20 and above. Studies have also declared that the process of aging has major alterations for Cardiovascular system and increased cardiovascular diseases prevalence with advancement of ages. Antioxidants are considered as important bioactive compounds due to many health benefits along with their pivotal role in delaying oxidative rancidity of numerous foods, Antioxidants are the substances that inhibit oxidation and can counteract the damaging effects of oxidation in body tissue, Herbal medicine is increasingly gaining acceptance from the public and medical professions due to advances in the understanding of the mechanisms by which herbs passively influence health and quality of life.

Keywords: Antioxidant, Cardio-protective, Cardiovascular Diseases, Oxidative stress.5 fluorouracil, Vitamin E.

INTRODUCTION:

Human heart is a vital organ without which survival is next to impossible [1], the cardiovascular system consists of heart & blood vessels which circulate blood throughout the body. It is responsible for transporting oxygen, nutrients, and hormones to body and removes cellular waste products from the body [2]. Cardiovascular diseases are group of disorders of the heart and blood vessels and groups of diseases that affect the heart and its parts [3]. Cardiovascular diseases are the number one cause of death globally [4]. The use of plants in therapy is certainly very old but currently it is experiencing a renewed interest among the population despite advances in modern medicine. According to the World Health Organization (WHO), more than 80% of the world population uses traditional medicine to cope with health problems [5]. Cardiovascular diseases (CVS) includes high blood pressure ,coronary heart disease ,congestive heart failure and stroke and account for 17,000,000 deaths per annum worldwide [6].Nature is the lifeline of our health since it provides all necessary things for survival, medicinal plants are nature's gift to human beings

to make disease free healthy life and play a vital role to preserve our health, although modern drugs are effective in preventing cardio-vascular disorders, their use is often limited because of their side effects. Nowadays, it is being realized that the herbs can protect the heart from heart diseases by their cardio-protective action [7,8] Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter [9].They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism [10].The oxidants or free radicals are species with very short half-life, highly reactive and possess damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either Oxygen derived, or Nitrogen derived. The most common reactive oxygen species include superoxide anion (O2), hydrogen peroxide (H2O2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy-nitrite anion (ONOO), Nitrogen dioxide (NO2) and Dinitrogen trioxide (N2O3) [11,12]. are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals [13].The antioxidant action has been ascribed to its ability to act chemically as a lipid based free radical chain-breaking molecule and thereby inhibiting lipid peroxidation through its own conversion into an oxidized product [14]. Vitamin E is a naturally occurring fat-soluble antioxidant which has been proposed as a treatment for both primary and secondary protection against cardiovascular events [15]. The antioxidant effect of vitamin E is not limited to a role of lipid phase Reactive Oxygen Species scavenger, as it can increase glutathione peroxidase activity [16]. Vitamin E is a group of eight lipophilic molecules, four of which are tocopherols and four of which are tocotrienols [17)]. And group of eight compounds (α-, γ-, β-and δ-tocopherols and -tocotrienols), which differ in their methyl substitution and saturation. The predominant form in the body, comprising over 90% of vitamin E, isα-tocopherol [18]. Vitamin E is an important nutrient with antioxidant and non-antioxidant functions, and certain evidence suggests that it has a cardiovascular protective role[19].

Classification of antioxidants

A) Antioxidants have been traditionally divided into two classes;

1) Primary or chain-breaking antioxidants and secondary or preventative antioxidants.

Chain-breaking mechanisms: L• + AH → LH + A• LO• + AH → LOH + A• LOO• + AH → LOOH + A•

Thus, radical initiation (by reacting with a lipid radical) or propagation (by reacting with peroxyl or alkoxyl radicals) steps are inhibited.

2) Secondary (preventative) antioxidants retard the rate of oxidation, e.g., transition-metal ion chelators may inhibit Fenton-type reactions that produce hydroxyl radicals:

$Fe_2^+ + H_2O_2 \rightarrow Fe_3^+ + \bullet OH + OH^-$

The chemical diversity of antioxidants makes it difficult to separate and quantify antioxidants from food/biological matrices where their combined action may be more relevant. Therefore, it is desirable to measure the TAC or the activity level directly from plant extracts andbiological fluids.

B) A basic classification of antioxidant assays based on the type of reaction:

(i) Hydrogen atom transfer (HAT)-based assays

(ii) Electron transfer (ET)-based assays.

Although the exact mechanism of chemiluminescent TAC assays is still debatable [20].

C) Based on mode of action, antioxidants can be classified into two main groups:-

I. Hydrogen atom transfer (HAT)

II. Single electron transfer (SET) assays [21].

Mode of action of antioxidants:

The cells which are most frequently damaged by oxidative stress are unsaturated fatty acids in lipids, cholesterol, different functional polypeptides, proteins and nucleic acids. Some antioxidants provide increased protection with increasing concentration, while others have optimal levels after which higher levels exert prooxidant effects[22, 23]. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc. Antioxidants play many vital functions in a cell and have many beneficial effects when present in foods [24, 25]. 5-Fluorouracil (5-FU) and its prodrug Capecitabine are widely used in the treatment of several solid tumours [26].5 Fluorouracil (5FU) is a fluoropyrimidine antimetabolite chemotherapeutic agent, which is used in the treatment of various solid cancers [27] , 5-FU also possesses several undesired cardiac toxicities, including coronary vasospasm, coronary thrombosis, cardiomyopathy [28] .

Potential mechanisms leading to 5-FU-related cardiotoxicity

Several mechanisms are thought to be responsible for 5-FU-related cardiotoxicity, some of which are inter-related. The two most likely contributors are ischemia and drug-related myocardial toxicity (Figure 1).

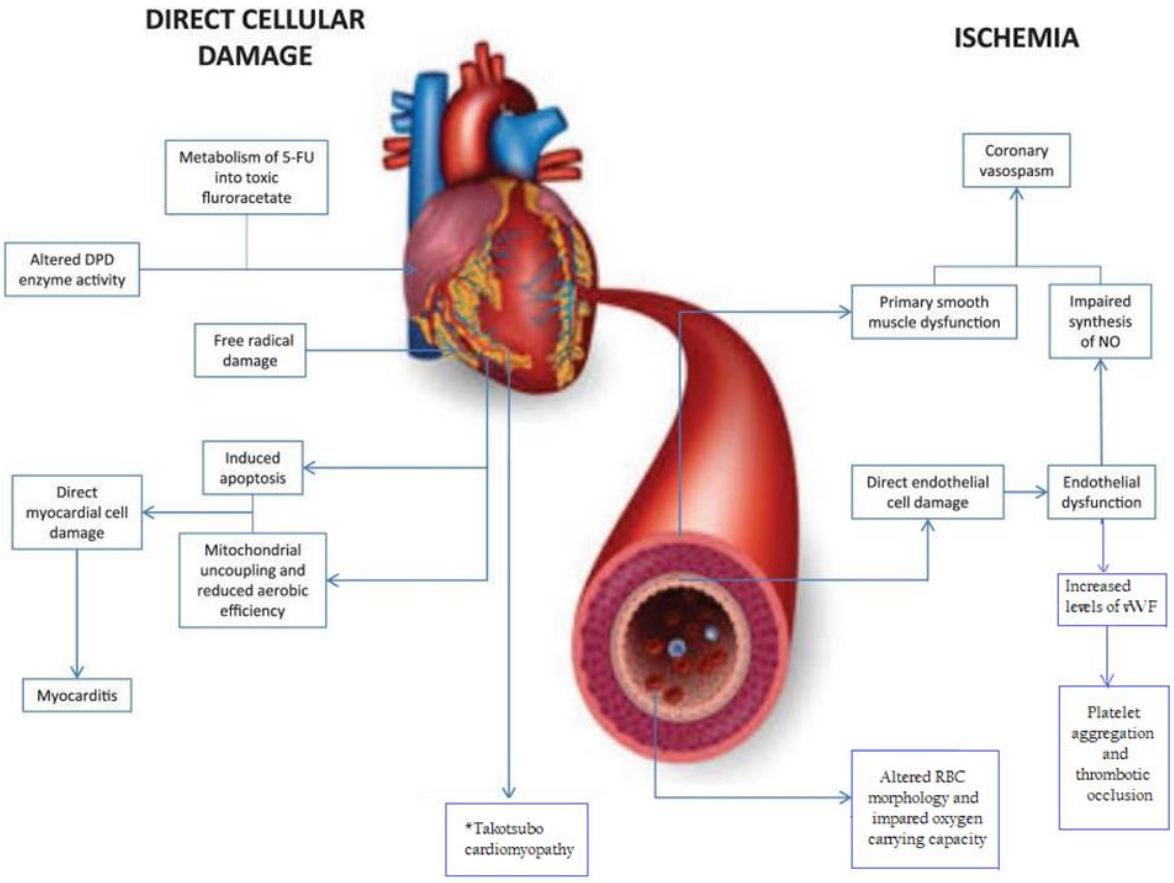

Figure:- 1.

Diagram outlining the two potential mechanisms by which 5-fluorouracil could lead to cardiotoxicity: direct cellular damage and ischemia.

(5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; NO, nitric oxide; RBC, red blood cell; vWF, von Willebrand factor. Takatsubo)

Cardiomyopathy is typically seen as a structural cardiomyopathic process, though there is some evidence which suggests that ischemia may contribute to the pathophysiology of this process. This remains a controversial area that requires further investigation [29]. 5-FU is in fact the third most commonly used chemotherapeutic agent in the treatment of solid malignancies across the world [30]. Herbal medicines having antioxidant properties, may therefore, have a protective role in cardiovascular disease [31, 32].*Hemidesmus indicus* (HI) family Asclepiadaceae) commonly known as Indian sarsaparilla or anantmool is a slender, laticiferous and twining shrub occurs over the greater part of India and some costal districts of Orissa. Several biological activities like hepatoprotective, anti-oxidant agent, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, anti-diabetics, nootropic etc [33, 34]. It mainly comprises saponins,

tannins, Hemidesmine, hemidesmol, hemidesterol, stearoptin , pregnone glycosides ,beta -sitosterol ,indicusin, coumarin, volatile oils ,triterpines , flavonoids[35].

Phytochemical analysis [36]

The qualitative analysis of plant extract indicated the presence of alkaloids, flavonoids, phenols and saponins in the roots of *Hemidesmus indicus* and tannins in leaves. Aqueous extracts in the present study were positive for alkaloids in contrast to Rajan et al. (2011). Review on *Hemidesmus indicus* also confirmed the presence of these compounds (Gayathri and Kannabiran 2009). The plant extracts were quantitatively analysed for secondary metabolites like phenols and flavonoids. In quantitative analysis of plant extract, the percentages of phenols are higher in aqueous extract (22.92 mg/100 gm) than flavonoids (4.23 mg/100 gm) and these results are like Sameera et al. (2010). Herbal medicines having antioxidant properties, May therefore have a protective role in cardiovascular diseases [37]. Several herbs and herbal products have been recommended for prophylactic and therapeutical effects in reducing cardiovascular disease (CVS) and that have been reviewed, [38] recent studies suggest that increase free radical formation and subsequent oxidative stress associated with the occurrences of a relative deficit in the endogenous antioxidants, maybe one of the mechanisms for the heart failure after myocardial infarction [39].

MATERIALS AND METHODS:

Experimental animals and diet

Animals:-

In-house laboratory bred healthy male rats of Wistar strain weighing 150-220gm were included for the study. Animals were housed in polypropylene cages. Animals were maintained under controlled temperature at $25^0C \pm 2^0C$ with 12hr light/dark cycle having access to food and water and libitum [40]. The experiments were carried out as per the guideline of CPCSEA, New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC) [41].

Source of Data:

Experiment was performed as per the standard bibliography, literatures and text books. The reputed journals and publications were obtained from college library and through web search.

Collection of plant Material:

The plant material (Leaves) was collected from the forest, nearby Tirupati.

Extraction:

Preparation of Extracts: Powdered leaves were subjected to successive extraction in a Soxhlet extractor with methanol. The extract obtained was concentrated in a rotary shaker evaporator to dryness to get a constant weight [42].

Drugs and Chemical:

All the drugs & chemicals are of pure analytical grade was obtained from the local suppliers.

- *-Hemidesmus indicus*
- -5 Fluorouracil
- -Vitamin-E

Vitamin E estimation

Into 3 stoppered centrifuge tubes (test, standard and blank) 1.5ml of each liver tissue extract was pipetted, plus 1.5ml of water respectively. To the test and blank 1.5ml of ethanol was added and to the standard 1.5ml of water was added. 1.5ml of xylene was also to all the tubes, stoppered, was mix well and centrifuged. Transfer 1.0ml of xylene layer into another stopper tube, taking care not to include any ethanol or protein, 1.oml of 2,2`~dipyridyl reagent was added to each tube, stopper and mix. Pipette out 1.5ml of the mixtures into spectrophotometer curettes and read the absorbance of test and standard against the blank at 460nm.Then in turn beginning with the blank, 0.33ml of ferric chloride solution was added. Mixed well and after exactly 15min read test and standard against the blank at 520nm.

Experimental design

Acute toxicity:

The acute toxicity study was performed by using up and down procedure (OECD-423, guidelines) [43].

MATERIALS AND METHODS

30 Wister male rats age 6-8 weeks weighing (150-200gm) were randomly divided into five groups of 6 each.

Group 1 consists of control animals. They received normal saline (5mg/kg Po) for 30 days.

Group 2 consists of rats treated with 5 fluorouracil 20mg/kg IP for 5 days.

Group 3 consists of 5 fluorouracil (20mg/kg IP for 5 days) and vitamin E (100mg/kg PO for 25 days).

Group 4 consists of 5 fluorouracil (20mg/kg IP for 5days) and low dose of Plant Extract for 25 days.

Group 5 consists of 5 fluorouracil 20mg/kg IP for 5days and high dose of Plant Extract for 25 days.

The animals were sacrificed by Pentobarbitone overdose, blood was collected, and the heart was isolated, and histology of heart was studied. The serum was separated immediately by cold centrifugation and was used for determination of the myocardial infarction marker enzymes LDH, CK-MB, AST, ALT, and ALP along with serum total cholesterol, triglycerides, LDL, and HDL. The enzymes, lipids and uric acid were estimated using commercial diagnostic kits.

Antioxidant study:

The free Radical scavenging activity in sodium nitroprusside/Greiss reagent system and inhibition of lipid peroxidation induced by iron ADP-ascorbate in liver homogenate and phenyl hydrazine induced haemolysis in erythrocyte membrane stabilization study. The extract was found to contain different levels of antioxidant properties in the models tested. In scavenging DPPH and superoxide radicals, its activity was found to be intense, while in scavenging NO radical, it was moderate. It also inhibited lipid peroxidation of liver homogenate and the haemolysis induced by phenyl hydrazine confirming the membrane stabilization activity[44].

i) Hydroxyl Radical Scavenging activity: About 60µl of ferrous chloride (1mM), was added to 90µl of 1,10-henanthroline (1mM). About 2.4ml of phosphate buffer saline (0.2 M, pH 7.4) was added to the mixture, followed by the addition of 150µl of hydrogen peroxide (0.17 M) and 1.5 ml of different concentrations of the extracts (10µg/ml - 100µg/ml). The mixture was then incubated for a period of 5 minutes at room temperature. All tests were performed in triplicate. The absorbance was read at 560 nm in a Double beam UV-visible Spectrophotometer (SYSTRONICS 2201) against blank (distilled water) [45].

ii) Nitric oxide radical scavenging: Sodium nitroprusside 5mM was prepared in phosphate buffer pH 7.4. To 1 ml of the various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5hrs after which, 0.5 ml of Griess reagent will be added. Absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicate (Sreejayan, 1996) [46].

iii) Superoxide scavenging: Alkaline DMSO was used as a super oxide generating system. To 0.5ml of different concentrations of the test compound, 1 ml of alkaline DMSO and 0.2ml of NBT 20mM in phosphate buffer pH 7.4 was be added. The experiment was performed in triplicate (Govindarajan, 2003) [47].

iv) DPPH-radical scavenging activity: DPPH Free Radical Scavenging Activity: The DPPH assay was based on the measurement of the scavenging ability of an antioxidant using the stable DPPH free radical. The free radical DPPH was purple in colour in ethanol and was reduced to the corresponding hydrazine, which was yellow in colour, when it reacts with a hydrogen donor. It was a discoloration assay, which was evaluated by the addition of the antioxidant to a DPPH solution in ethanol and the decrease in absorbance was measured at 490 nm.

% Inhibition = [(A0-A1) / A0 × 100]

Where A is the absorbance of the control [14] (blank, 0 without extract) and A is the absorbance in the 1 presence of the extract [48].

Processing of heart sample

Heart tissue were removed immediately and washed in ice cold physiological saline containing 0.9% sodium chloride. Heart tissue sample were homogenized in the appropriate buffer in a homogenizer and used for the following biochemical parameters.

Stastical analysis:

The data was expressed as ± SEM. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Turkey's test.

CONCLUSION

Hemisdesmus Indicus belongs to the family Asclepiadaceae commonly known as indian sarsaparilla. [49]. The present review reveals the importance of *hemidesmus indicus* in preventing and reversing the cardiovascular diseases and tries to compile some cardioprotective plants, Medicinal plants and their supplements can help in lowing the risk of cardiovascular diseases. *Hemidesmus indicus* contains various phytoconstituents belonging to the category glycosides, flavonoids, tannins, sterols and volatile oils, several studies have been carried towards its activities. It also protects radiation induced DNA damage with all these potentials this review explores the hidden potential and use of *hemidesmus indicus* and its benefit to mankind.Various phenolic extracts from plant sources have been shown to increase in AA with increasing concentration of the extract[50,51].The present review suggests that medicinal plants which possess good antioxidant potential are the best supplements for the diseases associated with oxidative stress,Animal studies have demonstrated gross pathological changes to cardiomyocytes in a dose-dependent fashion [52] as well as directly to endothelial cells, [53] which could represent the initial insult and subsequent 'reaction to injury' that leads to endothelial dysfunction. However, not all these pathological changes have been corroborated in human subjects experiencing symptoms of cardio-toxicity [54].

ACKNOWLEDGEMENT

Authors are thankful to the Department Of Pharmacology, Karnataka college of Pharmacy, Bangalore, India for providing necessary materials to carry out this study.

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