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Standardization and Cardio-Protective Effect of *Urtica dioica* against Doxorubicin Induced Toxicity in Wistar Rats

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ABSTRACT

Urtica dioica, belonging to genus Urtica L, family Urticaceae, commonly known as Bichhughass, is found to be growing throughout India is selected for the present study. This plant is used as mineral syrup of like Vitamin A, Vitamin C, Potassium, Manganese and Calcium. *Urtica dioica* is used as one of the important ingredients in most commonly used Ayurvedic preparation, named as "Herbal tea". The current study was designed with an aim to perform standardization as per WHO guideline and to perform cardioprotective activity of leaves of *Urtica dioica*. Phytochemical screening of the plant showed the presence of phytoconstituents like flavonoids, alkaloid, saponins, tannins and amino acids. TLC of extract of leaves shown spot in solvent system with O.98, 0.73 and 0.69 Rf value. The air dried leaves were coarsely powdered and was subjected to successive solvent extraction (SSE) with the help of soxhlet apparatus. The standardization of *Urtica dioica* leaves was performed as per the guideline of World Health Organization (WHO). The cardioprotective activity was performed using Wistar rats weighing about 180-200 gm. The biochemical analysis showed the levels of serum SGOT [(177.43±2.23)IU/L], SGPT [(54.12±12.21)IU/L], and LDH [(199.21±4.21) IU/L], were significantly increased in cardiotoxin treated group when it was compared to control group but total protein [(1.75±0.15)g/dl] level decrease when compared with control group. Administration of high dose (400 mg/kg) of ethanolic extract showed a reduction in the level of SGOT [(177.43±2.23) IU/L], SGPT [(54.12±12.21) UL/L] and LDH [(199.21±4.21) IU/L] when compared with liquid paraffin treated group but it showed the similar result as with doxorubicin and normal control group. Moreover, the level of total protein was [(4.6±0.2)] g/dl] were significantly increased when it was compared with liquid paraffin treated group but there was significant decrease when compared with normal control group. Ethanolic extract (200 mg/kg) treated animals showed individual heart tissue. Ethanolic extract (400 mg/kg) treated animals showed the heart tissue. So it can be concluded that the herb was potential attenuates and cardiotoxic effect.

INTRODUCTION

CARDIAC DISEASE

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels. It is estimated that 90% of CVD is preventable. Prevention of atherosclerosis is by decreasing risk factors through: healthy eating, exercise, avoidance of tobacco smoke and limiting alcohol intake. Treating high blood pressure and diabetes is also beneficial. Treating people who have strep throat with antibiotics can decrease the risk of rheumatic heart disease. The effect of the use of aspirin in people who are otherwise healthy is of unclear benefit. The United States Preventive Services Task Force recommends against its use for prevention in women less than 55 and men less than 45 years old; however, in those who are older it is recommends in some individuals. Treatment of those who have CVD improves outcomes.

Cardiovascular diseases are the leading cause of death globally. This is true in all areas of the world except Africa. Together they resulted in 17.3 million deaths (31.5%) in 2013 up from 12.3 million (25.8%) in 1990. Deaths, at a given age, from CVD are more common and have been increasing in much of the developing world, while rates have declined in most of the developed world since the 1970s. Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females. Most cardiovascular disease affects older adults. In the United States, 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD. The average age of death from coronary artery disease in the developed world is around 80 while it is around 68 in the developing world. Disease onset is typically seven to ten years earlier in men as compared to women.

Types

There are many cardiovascular diseases involving blood vessels. They are known as vascular diseases:

- Coronary artery disease
- Peripheral arterial disease
- Cerebrovascular disease

- Renal artery stenosis
- Aortic aneurysm

There are also many cardiovascular diseases that involve the heart.

- Cardiomyopathy
- Hypertensive heart disease
- Heart failure
- Pulmonary heart disease
- Cardiac dysrhythmias
- Inflammatory heart disease
  - Endocarditis
  - Myocarditis
- Valvular heart disease
- Congenital heart disease
- Rheumatic heart disease

Drug for Cardiac Disease

Research studies of heart failure have shown that several classes of drugs (medications) have shown to be the best for the treatment of heart failure. Heart failure patients may need multiple medications. Each one treats a different symptom or contributing factor. Each medication comes with its own instructions and rules. They can't do their job if you don't take them correctly. There are a variety of drugs prescribed for patients with heart disease. It is important for both patients living with heart disease and those who care for them to understand the prescribed medication, to follow the directions of usage, and to be able to recognize the possible side effects associated with the medicine. The drugs most commonly prescribed for heart disease include:

ACE Inhibitors: ACE inhibitors are a type of medication that dilates (widens) arteries to lower blood pressure and make it easier for the heart to pump blood. They also block some of the harmful actions of the endocrine system that may occur with heart failure.

Aldosterone Inhibitor: Eplerenone (Inspra) and spironolactone (Aldactone) and eplerenone are potassium-sparing diuretics. They can be prescribed to reduce the swelling and water build-up caused by heart failure. Diuretics cause the kidneys to send unneeded water and salt from the tissues and blood into the urine.

They may improve heart failure symptoms that are still present despite use of other treatments. These drugs protect the heart by blocking a chemical (aldosterone) in the body that causes salt and fluid build-up. This medication is used to treat patients with certain types of severe heart failure.

Angiotensin II Receptor Blocker (ARBs): ARBs are used to decrease blood pressure in people with heart failure. ARBs decrease certain chemicals that narrow the blood vessels so blood can flow more easily through your body. They also decrease certain chemicals that cause salt and fluid build-up in the body.

Beta-Blockers: Beta-blockers block the effects of adrenaline (epinephrine) and thereby improve the heart's ability to perform. They also decrease the production of harmful substances produced by the body in response to heart failure. They cause the heart to beat more slowly and with less force, lowering blood pressure.

Calcium Channel Blockers: Calcium channel blockers are prescribed to treat angina (chest pain) and high blood pressure. Calcium channel blockers affect the movement of calcium in the cells of the heart and blood vessels. As a result, the drugs relax blood vessels and increase the supply of blood and oxygen to the heart, while reducing its workload.

Captopril

It is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. Captopril was the first ACE inhibitor developed and was considered a breakthrough both because of its novel mechanism of action and also because of the revolutionary development process.[1] Captopril was discovered and developed at E. R. Squibb & Sons Pharmaceuticals based on concepts pioneered by Nobel Laureate John Vane and is now marketed by Bristol-Myers Squibb under the trade name Capoten.

Limitations

The adverse drug reaction (ADR) profile of captopril is similar to other ACE inhibitors, with cough being the most common ADR. However, captopril is also commonly associated with rash and taste disturbances (metallic or loss of taste), which are attributed to the unique thiol moiety. Captopril also has a relatively poor pharmacokinetic profile. The short half-life necessitates two or three times per day dosing, which may reduce patient compliance.

CARDIOTOXICANT

Cardiotoxicity occurs due to Smoking, High amounts of certain fats and cholesterol in the blood.

MECHANISM OF CARDIO-TOXICITY

Cardiotoxicity resulting from detrimental environmental insults has been recognized for a long time. However, extensive studies of the mechanisms involved had not been undertaken until recent years. Advances in molecular biology provide powerful tools and make such studies possible. We are gathering information about cellular events, signaling pathways, and molecular mechanisms of myocardial toxicological responses to environmental toxicants and pollutants. Severe acute toxic insults cause cardiac cell death instantly. In the early response to mild environmental stimuli, biochemical changes such as alterations in calcium homeostasis occur. These may lead to cardiac arrhythmia, which most often is reversible. Prolonged stimuli activate transcription factors such as activator protein-1 through elevation of intracellular calcium and the subsequent activation of calcineurin. Upregulation by activated transcription factors of hypertrophic genes results in heart hypertrophy, which is a short-term adaptive response to detrimental factors. However, further development of hypertrophy will lead to severe and irreversible cardiomyopathy, and eventually heart failure. From cardiac hypertrophy to heart failure, myocardial cells undergo extensive biochemical and molecular changes. Cardiac hypertrophy causes tissue hypoperfusion, which activates compensatory mechanisms such as production of angiotensin II and norepinephrine. Both further stimulate cardiac hypertrophy and, importantly, activate counter regulatory mechanisms including overexpression of atrial natriuretic peptide and b-type natriuretic peptide, and production of cytokines such as tumor necrosis factor-alpha. This counter regulation leads to myocardial remodeling as well as cell death through apoptosis and necrosis. Cell death through activation

of mitochondrial factors and other pathways constitutes an important cellular mechanism of heart failure.

CARDIOPROTECTIVE STUDY

Natural products and their active principles as source for new drug discovery and treatment of disease have attracted attention in recent years. Herbs are generally considered safe and proved to be effective against various human ailments. Herbal based therapeutics for cardiac disorders has been used in India for a long time and has been popularized in world over by leading pharmaceuticals. The currently observed rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors. All herbal products are safe and effective. The 21st century has seen a paradigm shift toward therapeutics evaluation of herbal products in cardiac disease models by carefully synergizing the strength of the traditional systems of the medicine with that of the modern concept of evidence based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy.

HERBAL MEDICINE

Herbs have been used as medical treatments since the beginning of civilization and some derivatives (eg, aspirin, reserpine, and digitalis) have become mainstays of human pharmacotherapy. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia. However, many herbal remedies used today have not undergone careful scientific assessment, and some have the potential to cause serious toxic effects and major drug-to-drug interactions. With the high prevalence of herbal use in the United States today, clinicians must inquire about such health practices for cardiac disease and be informed about the potential for benefit and harm. Continuing research is necessary to elucidate the pharmacological activities of the many herbal remedies now being used to treat cardiovascular diseases.

Since the beginning of human civilization, herbs have been an integral part of society, valued for both their culinary and medicinal properties. Herbal medicine has made many contributions to commercial drug preparations manufactured today including ephedrine from *Ephedra sinica* (ma-huang), digitoxin from *Digitalis purpurea* (foxglove), salicin (the source of aspirin) from *Salix alba* (willow bark), and reserpine from *Rauwolfia serpentina* (snakeroot), to name just a few. A naturally occurring β-adrenergic blocking agent with partial agonism has been identified

in an herbal remedy.[1] The recent discovery of the antineoplastic drug paclitaxel from *Taxus brevifolia* (Pacific yew tree) stresses the role of plants as a continuing resource for modern medicine.

PLANT PROFILE

Urtica dioica is originally from the colder regions of northern Europe and Asia, today this herbaceous shrub grows all over the world. Stinging nettle grows well in nitrogen-rich soil, blooms between June and September of every year, and reaches nearly 3 feet high. The stem is erect and green, the leaves are opposite, cordate at the base, oblong or ovate, finely toothed, dark green above and paler beneath. The flowers are in Reddish-brown to greenish-white color. The small, green, dioecious flowers occur as racemes in the axils of the upper leaves. Usually, the plant has either male or female flowers, in separate inflorescences, hence the specific name of the plant, dioica. *Urtica dioica* flowers from May to September every year.

Figure-1

Kingdom - Plantae

Subkingdom - Tracheobionta

Superdivision - Spermatophyta

Division - Magnoliophyta

Class - Magnoliopsida

Subclass - Hamamelidae

Order - Urticales

Family - Urticaceae

Genus - Urtica L

Common Name- Bichughass

CULTIVATION OF PLANT

Native range

Urtica dioica is native to tropical and subtropical India lists the following areas as native: Asia, China, Nepal America, Bhutan and Sri Lanka.

Global distribution

Urtica dioica is abundant in northern Europe and much of Asia, usually found in the countryside. It is less widespread in southern Europe and North Africa, where it is restricted by its need for moist soil, but is still common. In North America, it is widely distributed in Canada and the United States, where it is found in every province and state except for Hawaii, and also can be found in northernmost Mexico. It grows in abundance in the Pacific Northwest, especially in places where annual rainfall is high. The European subspecies has been introduced into North America and South America.

PLANT DESCRIPTION

Stem- Berect but weak and often supported by surrounding plants, generally 10-50+ cm (4-20+ in) tall, branching mainly from base.

Leaves- Opposite, triangular to heart-shaped in outline, bluntly and coarsely toothed, 1-6 cm (0.5–2.5 in) long, 1–4 cm (0.5–1.5 in) wide, reduced in size upward on stem, with linear bumps (cystoliths, concretions of calcium carbonate) on surface; leaf stalks (petioles) slender.

Flowers-Minute, unisexual (male and female in the same cluster), greenish; flower clusters more or less spherical 3–6 mm (0.1-0.2 in) wide, arising from leaf stalk-stem junction.

Fruit- Tiny and seed-like (achene), 1 mm (0.04 in) long, flattened, egg-shaped in outline, brown, enclosed by bract-like structures (calyx lobes).

CHEMICAL CONSTITUENTS

Stinging nettle is a powerhouse of nutrients. It contains on average 22% protein, 4% fats, 37% non-nitrogen extracts, 9-21% fiber, and 19-29% ash. The leaves contain about 4.8 mg chlorophyll per gram of dry leaves, depending on whether the plant was grown in the sun or shade. Surprisingly, more chlorophyll and carotenoids are found in plants that have been grown in the shade. The dried leaf of nettle contains 40% protein. They are one of the highest known sources of protein in a leafy green, and of superior quality than many other green leafy vegetables. The fresh leaves contain vitamins A, C, D, E, F, K, P, and B-complexes as well as thiamin, riboflavin, niacin, and vitamin B-6, all of which were found in high levels, and act as antioxidants. The leaves are also noted for their particularly high content of the metals selenium, zinc, iron, and magnesium. They contain boron, sodium, iodine, chromium, copper, and sulfur. They also contain tannic and gallic acids, gum, and wax.

REVIEW OF LITERATURE

Pradhan S, Manivannan S, (2015);

Intravenous injections of an aqueous extract of the aerial parts of the nettle, using two concentrations: 4 and 24 mg/kg/h resulted in a blood pressure drop of 15% and 38% proportionally to the administered dose. This decrease was correlated with an increase in diuresis and natriuresis. However, the hypotensive effect was reversible after one hour if a low concentration (4 mg/kg/h) had been used, while it persisted when using a high concentration (24 mg/kg/h).

Mihaljev E, (2014);

The anti-allergenic activity of the nettle is mainly due to two mechanisms. In addition to its inhibition of histamine H1 receptors, nettle inhibits tryptase, consequently reducing mast cell degranulation and the release of proinflammatory cytokine. In a randomized double-blind study with allergic patients having allergic rhinitis, an improvement in symptoms was observed after one week of treatment.

MATERIALS AND METHODS

-Identification, Collection and Authentication of the plant leaves

Fresh leaves of *Urtica dioica* were identified and collected in month of April from the herbal garden of Translam Institute of Pharmaceutical Education and Research, Meerut and then leaves of *Urtica dioica.*

-Drying and Size Reduction of Leaves

The collected leaves of *Urtica dioica* were air dried for 12 days in Pharmacognosy lab of TIPER, Meerut, Afterward, shade dried leaves of *Urtica dioica* were crushed and makes powdered with a mechanical grinder and passed through sieve no. 40. The sieved powder was stored in airtight container and kept in room temperature.

MACROSCOPICAL CHARACTERS

-**Size:** A graduated ruler in millimeter was used for the measurement of the length and width.

-**Colour:** Observed color of *Urtica dioica* leaves

-**Surface Characteristics:** The material was observed for surface characteristics and texture. To evaluate the cardioprotective activity of *Urtica dioica* leaves extracts in experimental rats.

-**Odour:** The material was checked for odour.

-**Taste:** The taste of leaves was observed.

PHYSICOCHEMICAL PARAMETERS OF *URTICA DIOICA* LEAVES

There are following parameters-

-Physical Constant

Ash Value

Total Ash Value

Acid Insoluble Ash Value

Water Soluble Ash Value

CHEMICAL TESTS FOR *URTICA DIOICA* LEAVES

Detection of cellulose-There are following

-Iodine test

-Sulphuric test

Detection of Chitin- There are following

-Iodine test

-Sulphuric test

-Phloroglucinol Test

-Safranin Test

Detection of Starch- There are following

-Iodine Test

-Water Test

Detection of Mucilage- There are following

-Iodine Solution And Sulphuric acid test

-Methylene Blue test

Detection of Protein Iodine Test- There are following

-Picric Acid Test

-Millon's Reagent Test

Detection of Fixed oil and Fats- There are following

-Ether Benzene and Chloroform Test

Detection of Tannins- There are following

-Ferric Chloride Test

Detection of Calcium Oxalate- There are following

-Acetic acid and Hydrochloride Test

Detection of Calcium Carbonate- There are following

-Acetic acid Test

PREPARATION OF EXTRACT OF *URTICA DIOICA* LEAVES

The dried powdered material was macerated for five days with ethanol with frequent shaking. The powdered leaves (350gm) were extracted using as Soxhlet extractor. This ethanolic extracts was concentrated to dryness under reduced pressure and controlled temperature (45-50ºC) to yield solid masses.

PHYTOCHEMICAL SCREENING OF LEAVE EXTRACTS

Ethanolic extracts of *Urtica dioica* were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, saponins, phenolics tannins and flavonoids.

Detection of Alkaloids

Extracts were dissolved individually in dil. HCl and filtered. The filtrates were used to test for the presence of alkaloids.There are following methods.

-Mayer's Test

-Dragendroff's Test

-Hager's Test

Detection of Carbohydrates- There are following methods.

Molish's Test

Conc. Sulphuric acid Test

Detection of Saponins- There are following methods.

Foam Test

Detection of Phenol- There are following methods.

Ferric chloride Test

Detection of Tannins- There are following methods.

Gelatin Test

Detection of Flavonoids- There are following methods.

Lead acetate Test

Zinc hydrochloric acid reduction Test

Detection of Amino Acids- There are following methods.

Millon's Test

Ninhydrin Test

EXPERIMENTAL ANIMAL

Wistar rats of (180-200 gm) of either sex were obtained from animal house facility of Translam Institute Pharmaceutical Education and Research. Animal were kept at room temp. 23±2ºC controlled humidity condition (50-55%) and 12 hr light and dark cycles. They were caged with a maximum of three animals in each polypropylene cage and were fed with standard food

pellets and water. The study was conducted after prior approval from the Institutional Animal Ethics Committee (IAEC) T.I.P.E.R, Meerut.

ACUTE TOXICITY

Acute toxicity was performed as per OECD guideline. Female rats were dosed in a stepwise procedure using the fixed doses of ethanolic extracts of *Urtica dioica* leaves 5, 50, 300 and 2000 mg/kg; and after dosing animals were observed for sign and conditions associated with pain, suffering, behavior and mortality, at first 30 min. and every 4 hr. periodically during the first 24 hr, and daily thereafter, for a total of 14 days.

CHRONIC TOXICITY

Female rats were dosed in a stepwise procedure using the fixed doses of ethanolic extracts of *Urtica dioica* leaves 50, 100, 200 and 300 mg/kg; and after dosing animals were observed for sign and conditions associated with pain, suffering, behavior and mortality, at first 30 min. and every 4 hr. periodically during the first 24 hr, and daily thereafter, for a total of 30 days.

INDUCTION OF CARDIOPROTECTIVE TOXICITY

Doxorubicin was used as cardiotoxin. Cardiac toxicity was induced in Wister rats with 1:1(v/v) mixture of doxorubicin in liquid paraffin, administered intraperitoneally at a dose of 2 ml/kg.

EXPERIMENTAL DESIGN FOR THE CARDIOPROTECTIVE ACTIVITY

ACUTE TOXICITY-

Animal were randomly divided into six groups of six animals each and treated orally, once daily for nine days in following manner.

Group I- Served as normal control and received distilled water (5ml/kg).

Group II- Served as toxic control and received distilled water (5ml/kg)and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) on 9^{th} day.

Group III- Served as standard group and received Silymarin(5mg/kg) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) on 9^{th} day.

Group IV-Treated with ethanolic extracts of *Urtica dioica* leaves (100mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kgi.p) on 9th day.

Group V- Treated with ethanolic extracts of *Urtica dioica* leaves (200mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) on 9th day.

Group VI- Treated with ethanolic extracts of *Urtica dioica* leaves

(400mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) on 9th day.

CHRONIC TOXICITY

Animal were randomly divided into four groups of six animals each and treated orally, once daily for 30 days in following manner.

Group I- Served as normal control and received distilled water (5ml/kg).

Group II- Served as toxic control and received distilled water (5ml/kg) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) for 30 days.

Group III- Treated with ethanolic extracts of *Urtica dioica* leaves (50mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) for 30 day.

Group IV-Treated with ethanolic extracts of *Urtica dioica* leaves

(100mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) for 30 days.

Group V- Treated with ethanolic extracts of *Urtica dioica* leaves (200mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) for 30 days.

Group VI- Treated with ethanolic extracts of *Urtica dioica* leaves (300mg/kg/day) and 1:1 (v/v) mixture of doxorubicin in a liquid paraffin (2mg/kg,i.p) for 30 days.

ESTIMATION OF HIGH DENSITY LIPID (HDL), LOW DENSITY LIPID (LDL) AND CHOLESTEROL

ESTIMATION OF HIGH DENSITY LIPID (HDL) VIA CHOLESTEROL

Method- Friedewald's Formula

In 1972 first described a formula for LDLc calculation using serum total cholesterol (TC), HDL-Cholesterol (HDLc) and triglycerides (TG) values. This formula became a landmark for LDLc estimation as an alternative to ultracentrifugation technique. This formula is:

$$LDLc = TC – HDLc - TG/5 \text{ (mg/dl)}$$

Clinical laboratories formerly measured HDL cholesterol by separating other lipoprotein fractions using either ultracentrifugation or chemical precipitation with divalent ions such as Mg^{2+}, then coupling the products of a cholesterol oxidase reaction to an indicator reaction. The reference method still uses a combination of these techniques. Most laboratories now use automated homogeneous analytical methods in which lipoproteins containing apo B are blocked using antibodies to apo B, then a colorimetric enzyme reaction measures cholesterol in the non-blocked HDL particles. HPLC can also be used. Subfractions (HDL-2C, HDL-3C) can be measured, but clinical significance of these subfractions has not been determined. The measurement of apo-A reactive capacity can be used to measure HDL cholesterol but is thought to be less accurate.

ESTIMATION OF LOW DENSITY LIPID (LDL) VIA CHOLESTEROL

Chemical measures of lipid concentration have long been the most-used clinical measurement, not because they have the best correlation with individual outcome, but because these lab methods are less expensive and more widely available.

The lipid profile does not measure LDL particles. It only estimates them using the Friedewald equation by subtracting the amount of cholesterol associated with other particles, such as HDL and VLDL, assuming a prolonged fasting state, etc.:

$$L \approx C – H – kT$$

Where *H* is HDL cholesterol, *L* is LDL cholesterol, *C* is total cholesterol, *T* are triglycerides, and k is 0.20 if the quantities are measured in mg/dl and 0.45 if in mol/l.

$$L = C – H – 0.16T$$

There are limitations to this method, most notably that samples must be obtained after a 12 to 14 h fast and that LDL-C cannot be calculated if plasma triglyceride is >4.52 mol/L (400 mg/dL). Even at triglyceride levels 2.5 to 4.5 mol/L, this formula is considered inaccurate. If both total cholesterol and triglyceride levels are elevated then a modified formula, with quantities in mg/dl, may be used.

This formula provides an approximation with fair accuracy for most people, assuming the blood was drawn after fasting for about 14 hours or longer but does not reveal the actual LDL particle concentration because the percentage of fat molecules within the LDL particles which are cholesterol varies, as much as 8:1 variation.

However, the concentration of LDL particles, and to a lesser extent their size, has a stronger and consistent correlation with individual clinical outcome than the amount of cholesterol within LDL particles, even if the LDL-C estimation is approximately correct. There is increasing evidence and recognition of the value of more targeted and accurate measurements of LDL particles. Specifically, LDL particle number (concentration), and to a lesser extent size, have shown slightly stronger correlations with atherosclerotic progression and cardiovascular events than obtained using chemical measures of the amount of cholesterol carried by the LDL particles. It is possible that the LDL cholesterol concentration can be low, yet LDL particle number high and cardiovascular events rates are high. Correspondingly, it is possible that LDL cholesterol concentration can be relatively high, yet LDL particle number low and cardiovascular events are also low. If LDL particle concentration is used to predict cardiovascular events, many other correlates of these clinical outcomes, such as diabetes mellitus, obesity and smoking, lose most of their predictive accuracy.

RESULTS

MACROSCOPICAL CHARACTERS

The leaves of Urtica dioica were observed for microscopical characteristics like color, odour, taste, size, surface and texture.

Table No. 1: Microscopical character of *Urtica dioica* leaves

Sr. No.	Organoleptic Parameter	Observation
1	Size	1-6 cm long, 1–4 cm wide
2	Colour	Greenish
3	Surface characteristics	texture
4	Odour	Odourless
5	Taste	Tasteless

PHYSIOCHEMICAL CHARACTER OF *URTICA DIOICA* LEAVES

Physicochemical characters like total ash, acid insoluble ash and water soluble ash value.

Total Ash value of *Urtica dioica a* leaves

The total ash value of the plant was calculated by subtracting total weight of crucible and weight of ash from weight of empty crucible. The total Ash value of Urticadioica was found to be 10.8%.

Table No. 2: Total Ash Value of *Urtica dioica a* leaves

Plant	Wight of Crucible (g) A	Wight of Drug (g) B	Wight of Crucible + Weight of Ash(g) C	Ash Obtained (g) (C-A)	Total Ash Value (%)
Urtica dioica leaves	28.89	2.15	31.04	0.210	21.0/2 = 10.5

Note- Results are the mean of three observation of drug sample.

Acid Insoluble Ash Value of *Urtica dioica* leaves

The acid insoluble ash of the plant was calculated subtracting total weight of crucible and weight of acid insoluble ash. The Acid insoluble ash value of *Urtica dioica* was found to be 1.4 %.

Table No. 3: Acid Insoluble Ash Value of *Urtica dioica a* leaves

Weight of crucible(g)	Weight of crucible(g) +drug(g)	Weight of crucible(g) + Total ash(g) A	Weight of crucible+ weight of acid insoluble ash (g) B	Acid insoluble ash Obtained (g) (A-B)	Acid Insoluble Ash (%)
28.68	30.68	28.89	28.86	0.028	1.4%

Note- Results are the mean of three observation of drug sample.

Water Soluble Ash Value of *Urtica dioica* leaves

The Water Soluble Ash of the plant was calculated by subtracting total ash from weight of crucible and weight of water soluble ash. The water soluble ash value of *Urtica dioica* was found to be 2.5 %.

Table No. 4: Observation for water soluble ash value of *Urtica dioica* leaves

Plant (leaves)	Weight of crucible(g)	Weight of crucible (g) + drug(g)	Weight of crucible(g) + total ash(g) A	Weight of crucible+ weight of Water soluble ash (g) B	Water soluble ash obtained (g) (A-B)	Water soluble ash (%)
Urtica dioica	28.68	30.68	28.89	28.84	0.05	2.5%

Note- Results are the mean of three observation of drug sample.

CHEMICAL TEST

Chemical test of *Urtica dioica* leaves were carried out to detect the presence of cellulose, chitin, starch, tannins, mucilage,, protein, calcium oxalate, calcium carbonate and fixed oils & fats.

Table No.5: Chemical constituents present in *Urtica dioica* leaves

Sr. No.	Constituents	Tests	Inference
1-	Cellulose	Iodine solution	+
		Iodine solution+Sulphuric acid	+
2-	Chitin	Iodine solution	-
		Iodine solution	-
		Iodine solution +Sulphuric acid	-
		Saffranin	-
3-	Fixed oil & Fats	Chloroform	-
4-	Tannins	Dil. Ferric chloride solution	+
5-	Starch	Iodine solution	+
6-	Mucilage	Iodine solution	+
7-	Protein	Iodine solution	+
		Alcoholic picric acid solution	+
		Millon's reagent	+
8-	Calcium carbonate	Acetic acid	+
9-	Calcium oxalate crystals	Powdered plant material± acetic acid	+

PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF *URTICA DIOICA* LEAVES

Phytochemical test were performed to find out the phytoconstituents present in the leaves of ***Urtica dioica*.**

Table-6 Phytochemical test of Ethanolic extract of *Urtica dioica* leaves

Chemical Test	Observation	Inference
Test of Alkaloids		
Hager's Test	Yellow color ppt is formed.	+
Mayer's Test	Yellow color ppt is formed.	+
Test for Phenolic Compounds		
$FeCl_3$	Deep blue-back color is formed.	+
Ferrous sulphate& Potassium Tartarate Test	Deep blue-back color is formed.	+
Test for Flavonoids		
Ferric chloride Test	Blackish red color is observed.	+
Magnesium Ribbon Tests	Magenta color is formed.	+
Test for Saponins glycosides		
Forth Foaming Test	Persistent foam is observed.	+
Foam Test	Foam persist for 10 min.	+
Test for Terpenoids		
Salkawski Test	Yellow color is formed in layer.	+
Test for Tannins		
Gelatin Test	White ppt is formed.	+

Note- +Sign indicate presence and –Sign indicate absence

SELECTION OF DOSES

The doses were selected on the basis of acute toxicity study as performed and reported, hence the dose, 200mg/kg & 400mg/kg which is about 1/10 and 1/5 of maximum tolerated safe dose for both extracts respectively.

DISCUSSION

The ethanolic extract (400 mg/kg,) showed maximum % inhibition i.e. 47.45 after 30 min while water extracts showed maximum % inhibition i.e. 40.67%, 32.53%, 31.9% and 28.57% respectively but standard drug Diclofenac sodium (15 mg/kg) showed 59.45%. From the above

result we can conclude that ethanolic extract (400 mg/kg) showed highest anti-inflammatory effect which was more similar to standard drug (Diclofenac sodium) as compare to aqueous extracts.

CONCLUSION

The present study reports the cardioprotective activity of *Urtica dioica* leaves extracts on doxorubicin induced cardioprotective in Wister rats. Phytochemical screening revealed the presence of glycosides, flavonoids, alkaloids, phytosterol, saponins and phenolics compounds in the extracts. Several investigators have shown that flavonoids are mainly responsible for cardioprotective potential in various experimental animal models. Thus it can be interpreted that the significant cardioprotective effect may be due to presence of flavonoids. On the basis of result obtained it can be concluded that administration (200mg/kg and 400mg/kg for both extracts) of *Urtica dioica* leaves extracts treated rats showed signification reduction of fats and lipids when compared with cardiotoxin and that level of total protein and fats were significantly increases.

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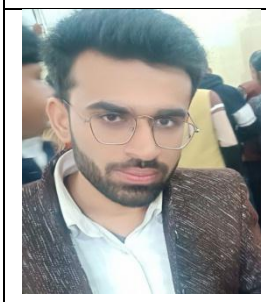 *Author -4*	***Author Name*** *Surya Pratap* *J.K INSTITUTE OF PHARMACY & MANAGEMENT* *KHURJA BULANDSHAHR, UTTAR PRADESH*