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RESEARCH ARTICLE

Pharmacological and Biochemical Evaluation of Cardioprotective Activity of Aqueous Leaf Extract of *Abutilon Indicum*in Isoproterenol Induced Myocardial Infarctionin Wistar Rats

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Conflicts of Interest: Nil

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ABSTRACT

Cardiotoxicity is a condition that occurs during therapy with several cytotoxic drugs and may be the dose limiting factor in the cancer therapy or imbalanced diet and lifestyle. The use of herbal supplements has become increasingly popular in recent years. Among all the cardio vascular diseases, Myocardial infarction is considered as one of the most dangerous disease. The treatment available may not be sufficient to treat the disease as it is caused by many factors to overcome the adverse effects caused by the synthetic medicine available. There is a need for the natural therapy with the help of medicinal plants.

The term cardiovascular disease [CVD] is very much familiar which commonly refers to a group of diseases that affects heart and its parts, whereas the term CVD mostly refers to MI [Myocardial infarction], angina pectoris, hypertension, stroke and other circulatory diseases..

Key words: CVD, Wistar Rats

Introduction

Cardiotoxicity is a condition that occurs during therapy with several cytotoxic drugs and may be the dose limiting factor in the cancer therapy or imbalanced diet and lifestyle. The use of herbal supplements has become increasingly popular in recent years. Among all the cardio vascular diseases, Myocardial infarction is considered as one of the most dangerous disease. The treatment available may not be sufficient to treat the disease as it is caused by many factors to overcome the adverse effects caused by the synthetic medicine available. There is a need for the natural therapy with the help of medicinal plants.

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Use of herbs and traditional systems of medicine is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of diseases.

From ancient time, plants have used as the major source of medicine and food for human being, and they have continued to provide mankind with new, novel therapeutic medicine and remedies. Since the last five decades, there has been a remarkably research in the study and use of herbal plants.

This current global interest in the study and use of medicinal plants has led to the characterization and identification of novel lead molecules, and isolation of active chemical compounds of therapeutic importance. The current world scenario of utilization of plant-

derived natural remedies hascreated a dire need for accurate and up to date information on the characteristic properties and therapeutic uses, efficacy, safety and quality of medicinal plant products.

The term cardiovascular disease [CVD] is very much familiar which commonly refers to a group of diseases that affects heart and its parts, whereas the term CVD mostly refers to MI [Myocardial infarction], angina pectoris, hypertension, stroke and other circulatory diseases. The common heart diseases that have been reported are coronary artery diseases, congestive heart failure, cardiac arrest, arrhythmias, and peripheral artery diseases.

It is known that number 1 cause of death globally is due to cardiovascular diseases because annually more people die from heart diseases than from any other grounds. Approximately 17.5 million people died from CVDs in the year 2012, representing 31% of all global deaths. Of these deaths, 7.4 million were due to coronary heart diseases and 6.7 million deaths were due to heart stroke.

Out of the 16 million deaths under the age of 60 due to non-communicable diseases, 85% are in low and middle income countries and 40% are caused by CVDs[1].

Cardiovascular diseases (CVDs).

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, myocardial infarction, congestive heart failure, stroke and congenital heart defects.

Types of cardio vascular diseases [CVD]

There are different types of cardio vascular diseases among them based on the prevalence of diseases across the world the most considerable CVD are like Atherosclerosis, Myocardial infarction, Ischemia, Cardiomyopathy[2, 3].

The medicinal plants are potential sources of drugs as they are rich in secondary metabolites and essential oils of therapeutic importance.

1. Uses of medicinal plants in various ailments are due to being economical, effective, their ease availability and due to their safety.
2. Because of these advantages the use of medicinal plants has been widely increased by the traditional medical practitioners in their day to day practice.
3. Foods are used commonly to meet our nutritional needs. However, foods obtained by plants contain a wide range of non-nutrient phytochemicals that are synthesized by plants for their own defence and for other biological functions. When we ingest these plant foods to meet our nutritional needs, we also ingest a wide variety of these non-nutrient phytochemicals. These phytochemicals have the potential for preventing chronic diseases and also non-toxic.
4. Cardiovascular disease is the number one cause of death globally and is projected to remain the leading cause of death. As many as 1.4 million children are suffering from heart related diseases in Pakistan and some 8,000 need heart surgeries annually, but out of them only 1,200 are operated upon. (Sixth “Biennial International Conference,” organized by the Pakistan Society of Cardiovascular and Thoracic Surgeries). Free radicals play deleterious role to body established ischemia. Presence of various antioxidant compounds in fruits and vegetables, for example, vitamins C and E, b-carotene and polyphenolics have been associated with decreased risks of several chronic diseases, such as coronary heart disease and some cancers. Antioxidants scavenging the free radicals and protect the body. There is inverse relationship between intake of polyphenols and heart diseases.
5. There is a large and increasing global burden of cardiovascular disease. Approximately 14 million individuals died of cardiovascular disease in 1990, and this is projected to rise to about 25 million by 2020.
6. The global burden of disease due to cardiovascular diseases (CVDs) is escalating, principally due to a sharp rise in the developing countries which are experiencing rapid health transition.
7. The continuous increase in incidences of cardiovascular disease is a manifestation of

chronic poor diet and lifestyle choices, which lead to diabetes and obesity.

8. More than 2000 plants have been listed in the Traditional (Herbal/Alternative) systems of medicine and some of these are providing comprehensive relief to the people suffering from cardio-vascular diseases, specially "hyperlipidemia" and "ischemic heart disease". WHO reports indicate that around eighty percent of the global population still relies on botanical drugs and several herbal medicines have advanced to clinical use in modern times. The use of Western medicinal drugs for the treatment of hypertension, congestive heart failure and post myocardial infarction are widely accepted.
9. Various phytoconstituents from plants were responsible for cardioprotective activity.

Pharmacology of cardioprotective plants: Phytoconstituents reported in cardioprotective plants significantly prevented the altered biochemical variation such as marker enzymes serum glutamate- pyruvate transaminase (SGPT) or alanine transaminase (ALT), serum glutamate oxaloacetate transaminase (SGOT) or aspartate transaminase (AST), creatinephosphokinase (CPK), alkaline phosphatise (ALP), lactate dehydrogenase (LDH), lipid profile including low density lipoprotein (LDL), VLDL (very low density lipoprotein), triglycerides (TGs), high density lipoprotein (HDL), total cholesterol and antioxidant parameters including Superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), Glutathione peroxidase (GPx), MDA (malonaldialdehyde) and glutathione reductase (GR) come to near normal status. Cardioprotective activity was evaluated using various pharmacological screening models like isoprenaline induced myocardial necrosis in rats, doxorubicin (DOX) induced cardiotoxicity in albino rats, cyclophosphamide induced oxidative myocardial injury in a rat model, ischemia-reperfusion-induced myocardial infarction in albino rats, cigarette Smoke-exposed Rats, adriamycin-induced cardio Myopathy in rats etc.[5-10]

MATERIALS AND METHODS[38-41]

MATERIALS

Drugs –

- Ketamine hydrochloride injection (Aneket from Neon Laboratories Limited)
- Isoproterenol (Samarth Life Science Pvt Ltd)
- Verapamil (VPL from Samarth Life Science Pvt Ltd.)

Chemicals-

Table 1: List of chemicals

S.No.	List of Chemicals
1	Hydrogen peroxide (H_2O_2)
2	5,5-Dithiobis 2-nitrobenzoic acid (DTNB)
3	Ether
4	Acetic acid
5	Thio barbituric acid
6	n-butanol pyridine
7	EDTA
8	Sucrose
9	Adrenaline
10	Trichloro acetic acid
11	Formalin
13	Potassium phosphate buffer
14	Sodium dodecyl sulphate
15	Ethanol
16	Sodium carbonate
17	Sodium hydroxide

20	Phosphate buffer
22	Formic acid
23	Hydrochloric acid
24	Sodium Nitroprusside
25	Chloroform
26	Sulphuric acid

Reagents will be prepared according to the need and some will be purchased from commercial sources.

Diagnostic kits: Diagnostic kits used for the estimation of marker enzymes CK-MB, LDH, SGOT and SGPT will be procured from **Span Diagnostic Ltd. India** and **AGAPPE Diagnostic**. **Instruments:**

- Micro centrifuge ("Microfuge" M/S Remi instruments Pvt. Ltd., Maharashtra, India).
- Semi Auto Analyser (MS 500 e, M/S Maysum technology Pvt. Ltd).
- ECG machine [Cardiart108DG (BPL)]
- Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India)
- Colorimeter (Systronics, Photoelectric Colormeter-112)
- Tissue homogenizer (M/S Remi instruments Pvt. Ltd.,Maharashtra, India)
- Autoclave

Result:

PHYTOCHEMICAL INVESTIGATION

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical tests were carried out for presence or absence of phytoconstituents like-Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Sterols, Anthocyanins, Terpenes and Tannins.

Table 1: Results of phytochemical tests of aqueous leaves extract of *Abutilon Indicum.*

S.NO	NAME OF THE TEST	CONCLUSION	
I	Tests for Carbohydrates Benedicts test Fehling's Test Molisch Test	 + + +	Carbohydrates were present the extract
II	Tests for Tannins and Phenolic Compounds 5% Fe Cl3 test Bromine water test Acetic acid solution test	 + + +	Tannins and Phenolic compounds were present in aqueous extracts
III	Tests for Alkaloids Mayer's Test Dragandraff's Test Wagner's Test	 + + -	Alkaloids were present in aqueous extract.

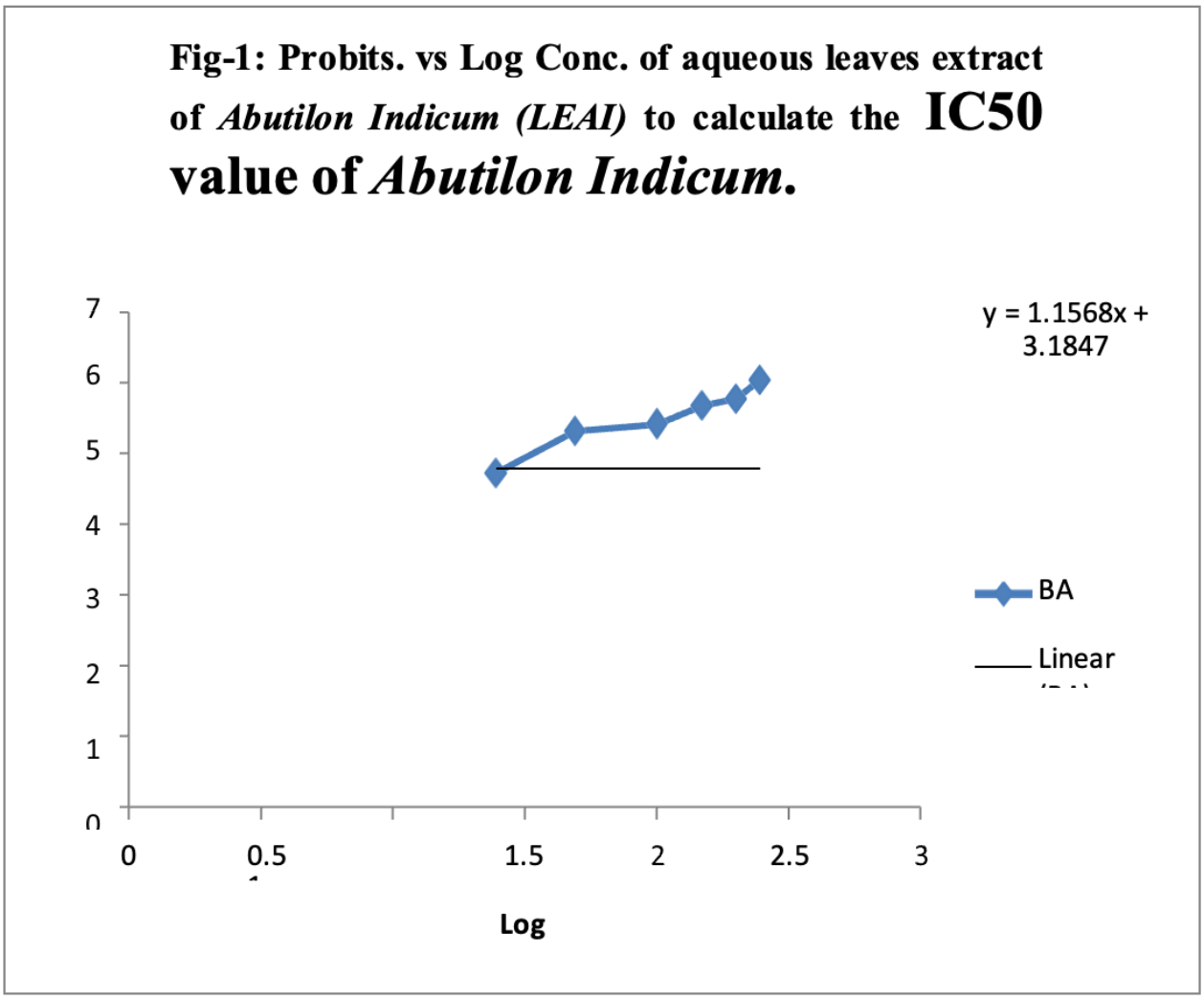
IV	Tests for Flavonoides Alkaline solution Ferric chloride test	 + +	Flavanoids were present in aqueous extract
V	Tests for Saponins Foam test	-	Saponins were absent in the extracts.

IN-VITRO PHARMACOLOGICAL INVESTIGATION

1. DPPH scavenging activity assay: *The crude extracts were screened for in-vitro anti-oxidant activity using DPPH radical scavenging method, Phosphomolybdenum reduction assay, Nitric oxide scavenging activity and reducing power assay and were compared with standard Ascorbic acid(A.A.).The aqueous leaves extract of Abutilon Indicum (LEAI) exhibited anti-oxidant activity in the scavenging of DPPH, Phosphomolybdenum reduction assay, Nitric oxide scavenging assay and Reducing power assay. The anti-oxidant potency of a compound was inversely proportional to the IC_{50} value. It was observed that a dose response relationship is found in the DPPH radical scavenging activity; the activity was increased as the concentration increased.*

Table 2: ***DPPH Scavenging Activity of Ascorbic Acid and Aqueous leaves extract of Abutilon Indicum (LEAI).***

S. No.	Groups	ncentration (µg/ml)	Log Conc.	%Scavenging activity (Mean ± S.E.M)	Probits of % Scavenging Activity
1.	A.A.	25	1.39	38.92 ± 0.0147	4.62
		50	1.69	63.19 ± 0.0435	5.21
		100	2.00	64.95 ± 0.0768	5.41
		150	2.17	75.45 ± 0.0216	5.67
		200	2.30	77.84 ± 0.0163	5.87
		250	2.39	84.97 ± 0.0321	6.14
2.	*LEAI*	25	1.39	20.86 ±0.5351	4.26
		50	1.69	36.16 ± 0.4475	4.51
		100	2.00	42.48 ± 0.3241	4.70
		150	2.17	47.87 ± 0.4326	4.85
		200	2.30	64.96 ± 0.5262	5.49
		250	2.39	72.82 ± 0.2471	5.65

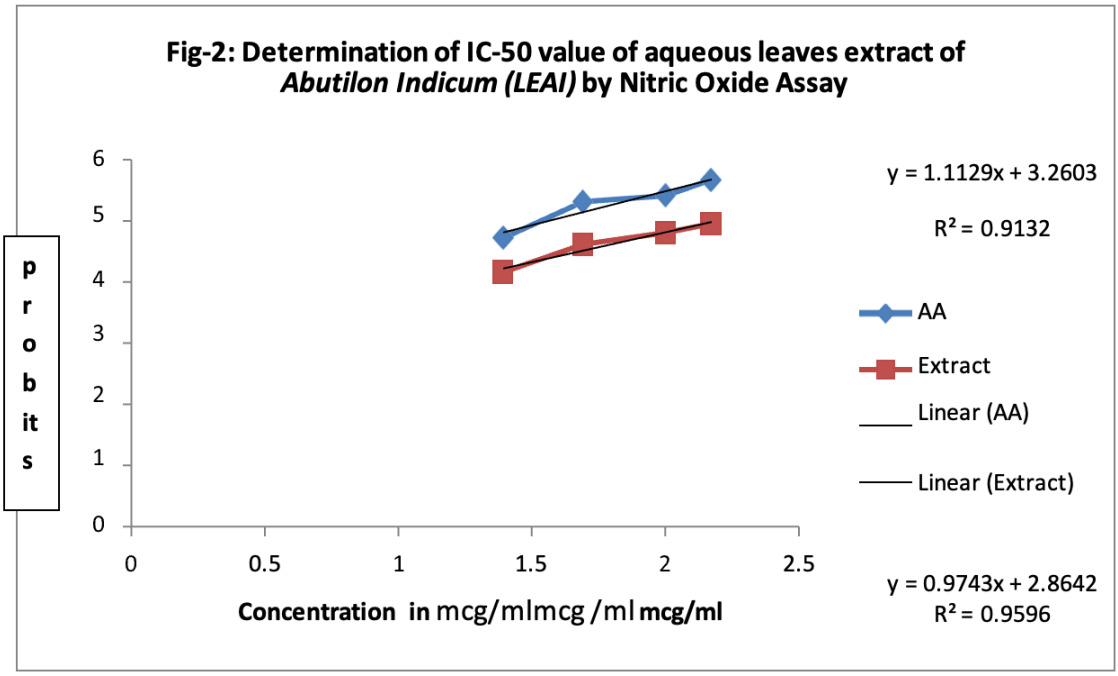


3. Nitric Oxide radical scavenging activity:

Nitric Oxide radical scavenging activity of plant extract with reference to A.A. has been tabulated in Table-. The crude plant extract exhibited anti-oxidant activity in the scavenging of nitric oxide radicals. The IC_{50} value of A.A. in the scavenging of nitric oxide radicals was 71.302µg/ml which has been shown in followingfig-

Table-3: Nitric Oxide radical scavenging activity of aqueous leaves extract of *Abutilon Indicum (LEAI)* with reference to Ascorbic Acid.

Concentration of solutions (mcg/ml)	Log conc.	% Inhibition by AA	Probits of % Inhibition by AA	% Inhibition by ***LEAI***	Probits of % Inhibition by ***LEAI***
50	1.39	32.4 ±0.43	4.53	29.35 ± 0.38	4.45
100	1.69	55.18 ±0.79	5.13	35.3 ± 0.29	4.61
150	2.00	61.72 ±0.58	5.31	46.02 ± 0.68	4.9
200	2.17	71.45 ±0.34	5.55	52.59 ± 0.42	5.08
250	2.30	83.5 ±0.24	5.95	58.43 ± 0.17	5.20



EVALUATION OF IN-VIVO CARDIOPROTECTIVE ACTIVITY

Isoproterenol Induced Myocardial Infarction

Albino Wistar rats either sex was divided into five groups (n = 6). The plant extract was treated for 30 days. At the end of the treatment period, animals of all groups were administered with isoproterenol at a dose of 85 mg/kg body wt., subcutaneously, twice, at an interval of 24 h.

Groups	Treatment
Group I	Normal Control
Group II	Isoproterenol (IP) (85 mg/kg, s.c.)
Group III	IP (85 mg/kg, s.c.)+ Plant Extract (100 mg/kg, p.o.)
Group IV	IP (85 mg/kg, s.c.)+ Plant Extract (250 mg/kg, p.o.)
Group V	IP (85 mg/kg, s.c.)+ Plant Extract (500 mg/kg, p.o.)

Myocardial Infarction (MI) is a major public health concern and common presentation of ischemic heart disease. It is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in necrosis of the myocardium. This is followed by numerous pathophysiology and biochemical changes such as lipid peroxidation, elevated levels of cardiac markers, altered lipid profile, etc. Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide. Isoproterenol-induced myocardial infarction is a standardized model to study the beneficial effects of many drugs and antioxidants.

In the present study, with the focus on the protective effects of aqueous leaf extract of *Abutilon Indicum*. Following isoproterenol administration, the heart weight increased significantly, with relatively unchanged body weight resulting in the increase of the heart weight to bodyweight ratio. Increase in heart weight might be attributed to increased water content, edematous intramuscular space and increased protein content. Pre-co-treatment of aqueous extract of *Abutilon Indicum* combination in IP injected rats significantly brings down the heart weight to body weight ratio indicative of its protection of myocardium against infiltration and it also could be due to

the decrease in water content of the myocardium.

Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the cellular membranes. Isoproterenol administration raised total cholesterol, TG, LDL cholesterol, VLDL and decreased HDL cholesterol level in the serum of Group 2 animals (Table no.9). Interestingly, treatment with aqueous leaf extract of *Abutilon Indicum*. Increased total cholesterol, LDL cholesterol and decrease HDL cholesterol are associated with raised risk for myocardial infarction. High level of circulating cholesterol, triglycerides and their accumulation in heart tissue are associated with cardiovascular damage. Hypertriglyceridemic patients at a risk for cardiovascular disease often develop a lipoprotein profile characterized by elevated triglyceride, dense LDL, and low HDL cholesterol, which causes myocardial membrane damage. Hypertriglyceridemia seen in isoproterenol treated rats is a condition observed in ischemic heart disease. The anti-hypertriglyceridemia activity of plant extract, signify that the myocardial membrane is protected against isoproterenol induced damage. Further, histopathological findings confirmed the induction of myocardial infarction by isoproterenol and the protection rendered by extracts treatment to the cardiac muscle. Histopathological examination of myocardial tissue in control illustrated clear integrity of the myocardial cell membrane and no inflammatory cell infiltration was observed. Isoproterenol injected rats showed coagulative necrosis, separation of cardiac muscle fibers and infiltration of inflammatory cells. The reduced inflammatory cell infiltration and normal cardiac muscle fiber architecture further confirmed the cardioprotective effect of aqueous leaf extract of *Abutilon Indicum*.

CONCLUSION AND SUMMARY

- The aim of present study was to evaluate cardioprotective activity of aqueous leaf extract of *Abutilon Indicum.*
- The acute oral toxicity study conducted for aqueous leaf extract of *Abutilon Indicum*indicates that this was safe up to 2000 mg/kg body weight.
- It can be concluded that pretreatment of aqueous leaf extract of *Abutilon Indicum* provides cardioprotection against isoproterenol induced myocardial infarction.
- This study validates traditional use of this extract for treating heart ailments thus confirming its folklore claim.
- The promising antioxidant efficacy of aqueous leaf extract of *Abutilon Indicum* was also demonstrated.
- The present study demonstrated the dose-dependent cardioprotective activity of aqueous leaf extract of *Abutilon Indicum* in a rat model of isoproterenol-induced Cardiotoxicity. Pretreatment with *LEAI* dose-dependently prevented cardiac injury as evidenced by serum biochemical analysis and heart histopathology. It protects heart from heart cell injury and antioxidant activity. This confirms the utility of the plant in folk medicine against Cardiotoxicity.
- In conclusion, *LEAI* is a potential cardioprotective agent against isoproterenol-induced Cardiotoxicity.

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