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Cardioprotective and Positive Inotropic Action of Seeds of *Linum usitatissimum* in Congestive Heart Failure

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Abstract : Congestive heart failure is, costly and deadly disease, is one of the leading causes of hospitalization and death worldwide. It is third most common cardio-vascular disease affect almost 2% of world population. Objective of present study is find out Cardioprotective and positive inotropic action of Seeds of *Linum usitatissimum* in Congestive Heart Failure. Methanolic extract and oil of seeds of *Linum usitatissimum*(MELU and OLU) were separated using soxhlet. Beneficial effects of methanolic extract and oil of seeds of *Linum usitatissimum* were carried out by doxorubicin (15mg/kg, i.p. within 3 weeks) to induce congestive heart failure in Wistar rat (*invivo* study). Following parameters like ECG recording, cardiac bio-markers like CPK-MB, LDH and SGOT and cytosolic Ca^{2+} level were estimated. In doxorubicin induced congestive heart failure both MELU and OLU showed significant increased in cytosolic Ca^{2+} level while significantly decreased QT interval as well as cardiac bio-markers. Present research work emphasizes that the seeds of *Linum usitatissimum* is beneficial in management of congestive heart failure.

Keywords: Cardioprotective, Inotropic Action, *Linum usitatissimum,* Congestive Heart Failure.

1. Introduction:

Congestive heart failure (CHF) is the common end-stage entity of many cardiovasculardiseases and it is defined as the inability of the heart to supply adequate blood in responseto systemic demands which is manifested by decreased exercise capacity, shortness ofbreath, fatigue and edema.[1]Congestive heart failure (CHF) is a growing health care burden that is associated withsubstantial morbidity, mortality and health care costs.[2] A recent study from Framingham suggests that the lifetime risk of developing CHF is approximately 20% for both men and women. Once CHF is diagnosed, the median survival with CHF is 1.7 years for men and 3.2 years for women.[3]

However, there have been dramatic advances in the pharmacological treatment of CHFover the past two decades. Currently, ideal management of CHF involves Angiotensin converting enzyme (ACE) inhibitors, Beta-blockers, diuretic and cardiotonics decreases rehospitalization of these patients.[4]But the major drawbacks of currently available and mostly used cardiotonics – digoxin have narrow therapeutic window and severe adverse effects on heart and other system. The other synthetic cardio tonics like Phosphodiesterase III inhibitors

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(Amrinone, Milrinone) have arrhythmias, thrombocytopenia, headache, fever, chest pain,hypokalemia like adverse effect while Dopaminergic drugs (Dopamine, Ibopamine) also possess some severe adverse drug effects like Atrial tachycardia, skin necrosis, Hypoprolactemia, Respiratory depressions. [5]

According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceedsthat of the conventional drugs by two to three times (Evans, 1994). Worldwide, herbalmedicine received a boost when the WHO encouraged developing countries to usetraditional plant medicine to fulfill needs unmet by modern systems.[6]

Linum usitatissimum is commonly known as flaxseed or linseed in English and Alshi in Guajarati belongs from Linaceae family. Seed of *Linum usitatissimum* contain Alpha-linolenic acid (ALA) which is precursor ofomega 3- fatty acid which has beneficial action on heart. It also contain lignanse specially secoisolariciresinol diglucoside (SDG), are claimed to be effective in reducing the risk of cardiovascular diseases and might be efficient in inhibiting the developmentof diabetes. Lignans – secoisolariciresinol diglycoside (SDG) and fiber mucilage whichhelp in obesity [7].

As per literature survey found that seeds of *Linum usitatissimum* may have cardio tonicactivity. It is reported in Ayurveda books that it possess cardiotonic activity.[8] Reviewarticles suggest that seeds of *Linum usitatissimum* can increase cAMP level by inhibition of phosphodiesterase enzyme (especially PDI -3) in blood platelets. Thus it may increasethe force of contraction in cardiac myocytes by inhibit phosphodiesterase enzyme andprove a good candidate as cardiotonic[9].

Linum usitatissimum also possesses **diuretic activity** [10] so it also helps to reducepreload and after load and thus it potentiate cardiotonic action. Additional due todiuretic action, it antagonizes hypertensive effect that is more prone for CHF. *Linum usitatissimum* possess anti-hypertensive[11] and anti hyperlipidemia[12] activities that might be beneficial to prevent cardiovascular complications. Additionally it alsopossesses anti-oxidant activity[13, 14] which is addition advantage of *Linum usitatissimum* seeds over other cardiotonics.

In light of above discussion *the objective of present study is to evaluate Linum usitatissimum having potent cardiotonic activity and having multiple actions that is beneficial in other cardiovascular complications and with least adverse effects.*

2. Materials and Methods:

The seed of *Linum usitatissimum* was acquired from "Anand Agriculture University" at Anand, Gujarat. Herbarium of the research drug was prepared and authenticated by Prof. Vishal Muliya, at Head of Botany Department, Christ College, Rajkot, Gujarat. India.

Methanolic extract of fine dried powder of seeds of *Linum usitatissimum* were prepared using soxhlet apparatus. Oil of seed was separated using soxhlet apparatus using petroleum ether as solvent.

All the experimental procedures and protocols used in study was reviewed andapproved by the Institution Animals Ethical Committee (IAEC) of School of Pharmacy,RK University, Rajkot, Gujarat and care of laboratory animals were taken as per the guidelines ofCommittee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA), Government of India.

Wistar rats of either sex initially weighing 220-280 gm were used for present study. Forinduction of congestive heart failure, doxorubicin were administered 1 mg/kg, i.p. for10 times in 3 weeks. The other groups were receiving their respective treatment alongwith doxorubicin. [15, 16]

The experimental animals were divided into five groups (n= 6).

Group 1 - Normal control: receive water only
Group 2 - Disease control: doxorubicin (1 mg/kg, i.p. for 10 times in 3 weeks)
Group 3 - Standard control: doxorubicin + digoxin (100 µg/kg/day, p.o.)
Group 4 - Test control A: doxorubicin + Methanolic extract of seeds of *Linum usitatissimum*(500mg/kg/day, p.o.)
Group 5 - Test control B:doxorubicin + oil of seeds of *Linum usitatissimum,* (0.6ml/kg/day, p.o.)

Study was carried out for a period of 3 weeks. ECG was recorded after 24 hours of last dose of doxorubicin. **NIBP-200** was used for recording and monitoring of ECG tracing. Mainly P wave, QRS complex, QT interval were observed and compared for all five groups.

Blood samples were allowed to clot for 30 min at room temperature. Then serums were separated using cooling centrifugation (REMI CM-12 cooling centrifuge). Serum samples were subjected for assessment Creatinine phosphokinase (CPK-MB), Lactate dehydrogenase (LDH), Serum glutamic oxaloacetic transaminase (SGOT) (using SPAN diagnostic kits). [17-20]

3. Statistical Analysis:

To check the significance of data, following statistical tests were performed:

ANOVA: to see the variability within all the groups.

Tuckey's test: for the same purpose mentioned in above test.

INSTAT software: to derive all the statistical terms like Standard Error of Mean (SEM),

ANOVA, P – value, Degree of freedom, Standard deviation, etc.

Data were considered statistically significant at $p < 0.05$ and highly significant at $p < 0.001$. Statistical analysis was performed using INSTAT statistical software.

4. Result and Discussion:

4.1 Cardioprotective effect in ECG study

Doxorubicin can cause cardiotoxicity which results in changes in ECG pattern and thusECG was monitored in all five groups. Doxorubicin cause prolongation of P wave, QRScomplex,QT interval, ST elevation and reduce cardiac cycles. In disease control group,QT interval was prolonged which is marker of hypertrophy of ventricles i.e. increasedventricular diameter.[21] The ST elevation may be observed due to cellular membranedamage occurred by oxidative stress.[22]

Test groups animals treated with MELU and OLU were reflected least prolongation of QT intervalsignificantly compared to disease control group. These findings suggests that MELU and OLU iseffective to avoid cardiac damage occurred by doxorubicin induced CHF model in wistar rats.

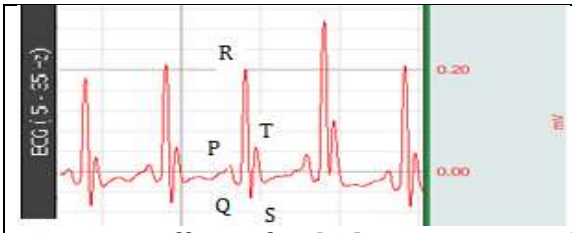

Figure I : Effects of Vehicle on QT Interval in ECG of Normal ControlGroup

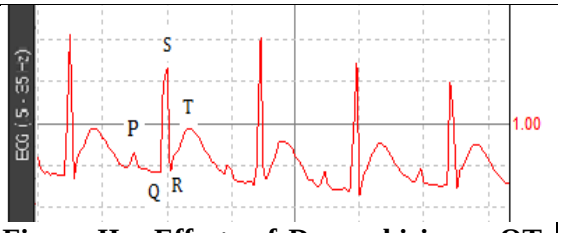

Figure II : Effects of Doxorubicin on QT Interval in ECG of DiseaseControl Group

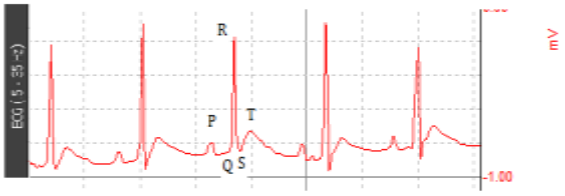

Figure III : Effects of Digoxin on QT Interval in ECG of Standard ControlGroup

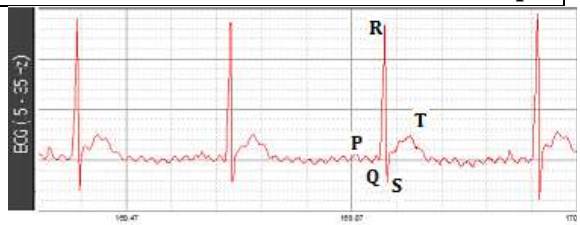

Figure IV : Effects of MELU on QT Interval in ECG of MELU Group

Figure V : Effects of OLU on QT Interval in ECG of OLU Group

Figure 1: Cardioprotective effect of *Linum usitatissimum* in ECG study using doxorubicin induced CHF model.

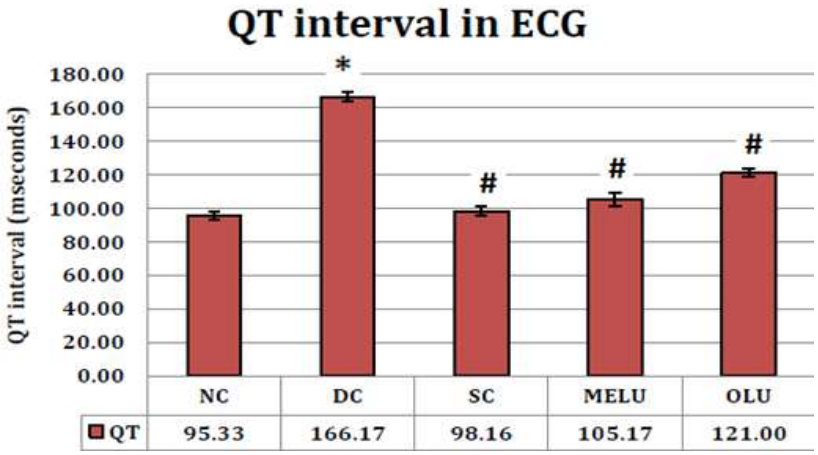

Figure 2: Beneficial Effects of Digoxin, MELU and OLU on QT Interval in Doxorubicin Induced Congestive Heart Failure

4.2 Effects of Digoxin, MELU and OLU on Bio-Markers (Cardiac Enzymes), Cytosolic Ca^{2+} level in Doxorubicin Induced Congestive Heart Failure Model

Disease control group, treated with doxorubicin alone, showed marked increased incardiac enzymes level i.e. CPK-MB, SGOT, LDH compared to normal control group. Thisincreased in enzymes level is might be due to deficiency of oxygen and glucose supply tomyocardial cells leading to rupturing of myocardial membrane and so that enzymesleaks out.[23, 24] Test groups showed significant decreased in these enzymes level whichsuggests that MELU and OLU may protect the myocardial cell membrane may be byincreasing supply of oxygenated blood to heart by increasing force of contraction.Disease control group, treated with doxorubicin alone, showed marked decrease incytosolic Ca^{2+} level compared to normal control group. This decreased in Ca^{2+} level maybe due to alteration in adrenergic pathway and alter the function of Na^{+} /K^{+} ATPase.Both test groups MELU and OLU showed marked increased in cytosolic Ca^{2+} level.

Table 1 Effects of Digoxin, MELU and OLU on Biomarkers in DoxorubicinInduced Congestive Heart Failure.

Groups	CPK-MB (U/L)	LDH (IU/L)	SGOT (IU/L)	Ca2+ (mg/dl)
Control	448.16 ± 9.56	202.16 ± 3.5	138.16 ± 4.67	2.03 ± .011
Digoxin	852.83 ± 4.73*	308 ± 18.18*	272 ± 4.92*	1.66 ± .023*
Standard Control	639.5 ± 8.03#	232.66 ± 1.3#	167.5 ± 3.79#	4.03 ± .03#
MELU	670 ± 3.88#	245 ± 2.12#	175.66± 3.27#	3.25 ± .04#
OLU	619 ± 10.16#	235.66± 1.54#	170.33 ± .76#	2.81 ± .03#
F *df* *P*	*350.89* *29(4, 25)* *<0.001*	*21.47* *29(4, 25)* *<0.001*	*180.46* *29(4, 25)* *<0.001*	*1122.89* *29(4, 25)* *<0.001*

n=6 and results were shown as mean ± SEM

* indicate significant difference in the data compared to **normal control** group and thelevel of significance was p<0.001 ≈ highly significant.
indicate significant difference in the data compared to **disease control** group and thelevel of significance was p<0.001 ≈ highly significant.

5. Conclusion:

Doxorubicin induced congestive heart failure model was used to determine methanolic extract and oil of seed of *Linum usitatissimum* MELU and OLU therapeutic effectiveness in CHF. MELU and OLU decreased QT prolongation. The cardiac enzymes like CPK, LDH and SGOT were also reduced by both MELU and OLU significantly, when compared with disease control group. Both MELU and OLU increased cytosolic Ca2+ level in 15 days Cardiac profilestudy.

These all results pointing towards the effectiveness of methanolic extract as well as oil of seeds of *Linum usitatissimum* in doxorubicin induced congestive heart failure (in vivo). Thus we can conclude that seed of *Linum usitatissimum* are effective in treatment of congestive heart failure.

6. Recommendation for Further Study:

Further research is required to find out mechanism of action responsible for its cardiotonic activity and isolation, characterization and purification of active constituent which is responsible for cardiotonic activity. Future aspects of this study include preparation of suitable formulation as well

as bulk manufacturing of same at industrial scale.

7. Acknowledgement

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