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PROTECTIVE ROLE OF *CISSUS QUADRANGULARIS* LINN. IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS

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ABSTRACT

In the present scenario cardiac disease is becoming a problem countered by almost every section of mankind. By 2020, heart disease and stroke will become the leading causes of both death and disability worldwide, and the mortality rate may increase to more than 20 million a year. Several plant products are known to exhibit creditable medicinal properties for the treatment of cardiac ailments. This study was designed to investigate the cardioprotective effect of *Cissus quadrangularis* Linn. on isoproterenol induced myocardial injury in rats with respect to cardiac markers, antioxidant defense system and serum lipid profiles. Animals were grouped into six comprising of six rats each. Group 1 served as normal control. Group2 served as the disease control induced with isoproterenol (20 mg/kg, s.c., twice at 24 h intervals at the end of the experimental period). Group-3 was pre-treated with *C.quadrangularis* extract (100mg/kg) for 30 days and induced with Isoproterenol Group-4 was pre-treated with *C.quadrangularis* extract (200mg/kg) for 30 days and induced with Isoproterenol Group-5 *C.quadrangularis* Linn extract alone (200mg/kg) for 30 days. Isoproterenol (ISO) administration induced a significant increase in serum aspartate transaminase, alanine transaminase, lactate dehydrogenase, and creatine kinase with a concomitant decrease in their activity in heart tissue. Isoproterenol treated rats showed a significant increase in the levels of total cholesterol, triglycerides and phospholipids in serum. The reversal of marker enzymes to near normalcy was observed in the *C.quadrangularis* Linn. treated groups. Pre-treatment with *C. quadrangularis* Linn. extracts showed a significant effect and maintained the levels of lipids in serum.

Keywords: *Cissus quadrangularis*, Isoproterenol, Cholesterol, Triglycerides, Marker enzymes.

INTRODUCTION

The enormous resources available in India coupled with their biodiversity can be explored to unravel biological potentials which could be used as "green medicines" to treat various life threatening diseases [(1)]. Herbal medicines are gaining global acceptance due to the advancement in understanding the mechanism of action of herbs [(2)]. Myocardial infarction (MI) is one of the leading causes of mortality[3] and MI could trigger complex impact on the biochemical functioning heart[4] and is expected to occupy the first position as a killer disease by 2020[5].

Although existing modern drugs can help in the management of heart diseases they are bound to cause side effects[6] and plant sources are emerging as alternate therapy for reducing the risks of cardiac problems. *Cissus quadrangular a plant from* Vitaceae family has been traditionally used as analgesic and for healing broken bones[7]. The favourable effects of the new dugs on the heart have been analysed using different methodologies[8]. Because of the effects of infarct-like necrosis of the heart muscle through isoproterenol, a β-adrenergic agonist[9] and this is used as a validated model for human MI[10].

The present communication is therefore aimed at deducing the cardioprotective effect of the *C. quadrangularis* Linn. leaf extract on induced myocardial necrosis by analysing marker enzymes in the serum and heart .

MATERIALS AND METHODS

Plant collection and extraction

Plant source selected for the present study was *Cissus quadrangularis* **Linn.** Collection of aerial parts of the selected plant was from Trichy region identified nad authenticated using Gambles Flora of Presidency of Madras.

200gm of coarsely powdered shade dried *Cissus quadrangularis* Linn. was mixed with 1200 ml of water, boiled and reduced to one third volume. The filtrate obtained in paste form was subjected to pre-clinical screening.

Animals

Standard protocols were followed using adult albino rats for the studies after getting appropriate ethical guidelines.

Experimental protocol

Animals were divided into five groups of five rats (both sex) each. The experimental design given below has been followed for the present study.

Group I: Normal control

GroupII: Disease control received subcutaneous injection of isoproterenol (20mg/kg, b.w) at the conclusion of experimental period for 2 consecutive days

Group III: Rats pretreated with *Cissus quadrangularis* Linn 100mg/kg body weight for 30 days and induced with standard drug at the mentioned dose on the 29th and 30th days.

Group IV: Rats pretreated with *Cissus quadrangularis* Linn 200mg/kg body weight for 30 days and induced with isoproterenol at the mentioned dose on the 29th and 30th days.

Group V: Normal rats received aqueous extract of *Cissus quadrangularis* Linn 200mg/kg b.w for 28 days

Sacrificing the animals after the experimental period was done by cervical decapitation. Serum was separated from blood and heart was dissected, washed and homogenized using buffers which was used then for studying various parameters.

Assay of Aspartate transaminase [11]

The assay mixture containing 1ml of compound and 0.2 ml of serum was incubated for 1 hr at 37^{o}C with controls using serum in which the reaction was arrested by the use of DNPH. Addition of 0.5 ml of NaOH to the samples after keeping for 30 min at room temperature resulted in colour development which was analysed at 540 nm.

Assay of Alanine transaminase [11]

The above protocols were repeated with the use 5 ml of NaOH instead of 0.5 mL NaOH.

Assay of Lactate Dehydrogenase [12]

Samples were analysed for lacate dehydrogenase assay using 1.0 ml of the buffered substrate by following protocols reported in ref. 12.

Assay of Creatine Kinase [13&14]

Creatine Kinase assay was carried out using methods described in ref 13 and 14.

Estimation of Total Cholesterol [15]

Total Cholesterol was estimated using methods reported in ref 15.

Estimation of Phospholipids [16]

Phospholipids estimation was done using reported methods described in ref 16.

Estimation of Triglycerides [17]

Triglycerides estimated by protocols referred in ref 17.

Statistical analysis

The experimental results expressed were: mean ± S.E.M. ANOVA and t-test were performed and statistically significant was taken for $p<0.05$.

RESULTS

The results obtained **(Table 1)** clearly indicate a significant increase in serum cholesterol, TG and PL in the standard drug-induced rats. The animals were pretreated with the plant extract (Group III & GroupIV) showed a decrease in serum cholesterol, TG and PL compared with disease control. The Group V animals did not show any marked variation in the serum lipid profile. The results indicate the cardioprotective activity in the selected plant.

Table 1: Effect of the plant drug on the serum lipid profile in Isoproterenol induced MI

Groups	I	II	III	IV	V
Cholesterol (mg/dl)	68.51 ± 1.68	132.41± 1.34*	84.71 ± 0.91	63.2 ± 1.11**	71.2 ± 1.03#
Phospholipid (mg / dl)	10.52 ± 0.91	29.63 ± 0.83*	16.32 ± 0.44	11.26 ± 0.52**	14.0 ± 1.13#
Triglycerides (mg/dl)	156.25±1.42	312.5±2.31*	181.25±1.04	156.25±0.98**	157.5±1.43#

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with normal control
**p<0.05 statistically significant when compared with disease controlgroup
# p<0.05 statistically non-significant when compared with normal group

The results obtained **(Table 2)** clearly indicate a significant increase in AST, ALT, LDH and CK in the isoproterenol-induced rats. Pretreatment with the plant extract (Group III & Group IV) to animals showed a decrease in serum marker enzymes, which was comparable to the disease control. The Group V animals did not show any marked variation in the serum marker enzymes level. The results indicate the cardioprotective activity in the selected plant

Table 2: Effect of the plant drug on the serum marker enzyme activity

Groups	I	II	III	IV	V
Serum AST (IU/L)	17.46±0.56	104.76±0.91*	46.56±1.09	20.32±0.38**	15.0±0.44#
Serum ALT (IU/L)	22.38±0.99	82.9±1.01*	45.22±0.71	25.31±0.61	20.91±0.43#
LDH (IU/L)	80.33±1.41	157.21±0.89*	103.47±1.03	84.13±0.78**	79.34±0.81#
CK (IU/L)	273.22±10.31	681.9±3.89*	352.31±1.92	282.11±4.91**	266.67±3.22#

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with normal control
**p<0.05 statistically significant when compared with disease controlgroup
# p<0.05 statistically non-significant when compared with normal group

The results obtained **(Table 3)** clearly indicate a significant decrease in tissue LDH and CK in the standard drug-induced rats. Pretreatment with the plant extract (Group III & Group IV) to animals showed a higher level of LDH and CK, which was comparable to the normal control. In group V animals there was no significant variation in the LDH and CK level. The results indicate the cardioprotective activity in the selected plant

Table 3: Effect of the plant drug on tissue LDH and CK in experimental animals

Groups	I	II	III	IV	V
LDH (IU/L)	87.78±6.3	52.72±0.39*	76.54±0.66	85.99±1.96**	84.3±2.96#
CK (IU/L)	22.31±1.09	5.37±0.97*	12.3±1.11	19.91±0.83**	21.9±1.21#

Values are mean ± SEM (n=6)
*p<0.05 statistically significant when compared with control group
**p<0.05 statistically significant when compared with disease control group
# p<0.05 statistically non-significant when compared with normal group

DISCUSSION

Isoproterenol induction increases the biosynthesis of cholesterol with a cocurrent decrease in its utilization. Free radicals are liberated in excess on induction with isoproterenol which might also be a reason for the accumulation of cholesterol in tissues. This may also lead to a decrease in the rate of ester hydrolysis of cholesterol and also reduces the efflux of cholesterol. Pretreatment with the plant extract restored the level of cholesterol. [18]

The increased phospholipids content in drug induced rats may be due to greater degradation, due to the injury caused in the cardiac tissue. The phospholipids content was close to normal levels in the animals given treatment with *C.quadragularis* Linn. Due to its membrane stabilizing activity, the plant extract might have may induced myocytes to regenerate new phospholipids which necessary to repair the damaged membrane [19].

Hypertriglyceridemia was observed in the isoproterenol treated rats which may be due to the inactivation of the lipoprotein lipase in the myocardium increased accumulation of the triglycerides in circulation. The inactivation of the hydrolytic enzyme results in the accumulation of ester cholesterol which in turn produces myocardial membrane damage. Pretreatment with the *C.quadragularis* Linn plant extract maintains the activities of LCAT (Lecithin: cholesterol acyl esterase), lipo protein lipase, and cholesterol ester synthetase (CES). The treatment also maintains the HDL levels in serum and decreases the TG and cholesterol content,

indicating that *C.quadragularis* Linn could be used as a lipid lowering agent[20].

The myocardium is rich in enzymes (AST, ALT, CK and LDH) which are required for its metabolic activity. These enzymes serve as markers of functioning of the heart. Drug induced damage destabilizes the membrane and these enzymes leak into the extra-cellular fluid [21] In the isoproterenol induced rats, the activity of ALT, AST, LDH and CK has been increased in serum while a significant reduction of LDH and CK was noticed in the cardiac tissues. The onset of myocardial necrosis is confirmed with these biochemical changes [22]. Pretreatment with *C.quadragularis* Linn showed the normalization of the activity of diagnostic marker enzymes when compared with isoproterenol treated rats, indicating the antioxidant potential of *C.quadragularis* Linn, which protects heart from lipid peroxidative damage.

CONCLUSION

From the present results, it may be concluded that *C.quadragularis* Linn is possess cardioprotective potentials, which could be used to treat different cardiac problems.

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