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Unmasking the therapeutical potential of *Cuscuta reflexa* against isoproterenol-induced cardiotoxicity in rats

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INTRODUCTION & AIM

Cardiotoxicity, especially Myocardial infarction, is a manifestation of coronary artery disease due to lack of oxygen, which causes coronary microvascular dysfunction, inflammation, atherosclerosis, and vasospasm.

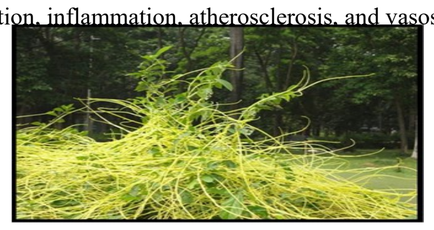

Fig. 1 *Cuscuta reflexa*

Cuscuta reflexa, the dodder plant, belongs to the Convolvulaceae family (Fig. 1). It is common throughout India. Phyto-chemistry of *Cuscuta reflexa* that it constitutes of carotenoids, sitosterol, caffeic acid, mannitol, flavonol, dulcitol, quercetin, tannins, lutein, lycopene, kaempferol, coccinoside B, kaempferol-3-O-glycoside, stigmasterol, cystamine, quinic acid, 6,7-dimethoxy Coumarin, cuscutalin, isorhamnetin-3-O-neohesperidoside, myricetin, abscisic acid, reflexin, bergenin, and some other polyphenol. Since *Cuscuta reflexa* contains flavonoids, they are of great therapeutic value owing to their antioxidant, anti-inflammatory, antiviral, anticancer, and anti-ageing properties. Our study hypothesis has been designed to evaluate the effect of *Cuscuta reflexa* on myocardial toxicity by evaluating secondary metabolites, Acute oral toxicity, cardiac markers, biochemical parameters, an Electrocardiogram, antioxidants, and histopathological analysis.

METHOD

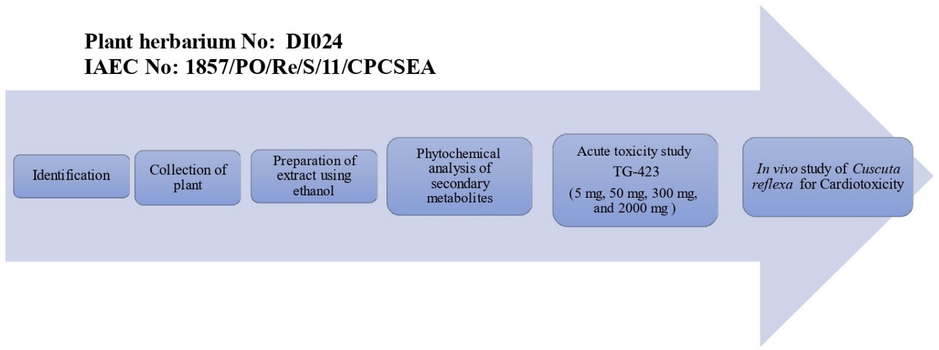

Fig. 2 Flow of methodology

Isoproterenol-induced Myocardial Infarction

The animals were grouped into five groups, containing six animals in each group. Group I and Group II were termed Normal control and Disease Control groups. Group III was administered with Standard Metoprolol. Group IV & V were treated with low and high doses of CREE (100 mg/kg & 200 mg/kg), respectively. All the treatments were given for 21 days through the oral route. Apart from the standard control, all other groups were subjected to Isoproterenol (ISO) (85mg/kg) toxicity for the last two days. Elevated levels of serum biomarkers confirmed MI.

Recording of Electro-Cardiogram

After 24 hours of previous treatment, the rats were subjected to electrocardiographic (ECG) parameters. The rats were anaesthetised with diethyl ether, and the ECG readings were recorded using bipolar limb lead (I, II, III) from a rat lying straight on the back with the front legs semiflexed and hind legs extended slightly. Electrodes were then subcutaneously attached to the front and rear legs; the chest lead was subcutaneously attached near the heart towards the left ventricle. The charges were connected with a digital data acquisition system (AD instruments, Power Lab 4/26) at the intensity of 5 mV with a graph speed of 100mm/sec. The electrocardiographic parameters such as heart rate, QT, RR, ST, PR and QRS intervals were measured. Estimation of serum biomarkers, antioxidants, and histopathological analysis was carried out.

RESULTS & DISCUSSION

Preparation of extract

Cuscuta reflexa was extracted using coarse powder, Soxhlet, and ethanol as the solvent. When subjected to extraction, 250gm of coarse powder yielded 12.01gm of extract. Therefore, the percentage yield was found to be 4.804%.

Phytochemical analysis

The phytochemical analysis of the whole plant extract of *Cuscuta reflexa* showed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, tannins, flavonoids, and steroids.

Effect on Electrocardiographic Parameters

Table 1: Effect of CREE on heart rate, QT, RR, ST, PR, and QRS intervals.

Groups	Heart rate (beats/min)	QT interval (ms)	RR interval (ms)	ST Height (ms)	PR interval (ms)	QRS interval (ms)
Normal control	314.36±3.66	69.43±1.56	188.0±4.56	12.33±0.93	32.55±1.25	14.0±1.23
ISO control (85mg/kg)	426.21±5.42***	88.24±1.72***	140.0±3.21***	31.5±2.01***	41.35±2.31***	20.0±1.56***
MET,50mg/kg	333.66±3.78$^{**\#\#\#}$	71.74±2.31$^{\#\#\#}$	182.0±3.58$^{\#\#\#}$	14.70±1.18$^{\#\#\#}$	34.1.1±1.32$^{***\#\#\#}$	15.23±2.11$^{\#\#\#}$
CREE,100mg/kg	410.33±1.52$^{***\#\#}$	83.79±3.34$^{***\#\#}$	149.0±4.89$^{***\#\#}$	28.53±1.13$^{***\#}$	39.24±2.54$^{***\#\#\#}$	17.20±1.96$^{**\#}$
CREE,200mg/kg	372±4.35$^{***\#\#\#}$	73.28±3.37$^{**\#\#\#}$	174.5±6.55$^{***\#\#\#}$	26.55±2.08$^{***\#\#\#}$	36.10±2.31$^{***\#\#\#}$	16.55±1.48$^{*\#\#}$

All values are mean ± SEM, n=6, $^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05 when compared to normal control; $^{\#\#\#}P$<0.001, $^{\#\#}P$<0.01, $^{\#}P$<0.05 compared to ISO control. ISO-85 (Isoproterenol-85mg/kg), MET-50 (Metoprolol-50mg/kg), CREE-100 (*Cuscuta reflexa* ethanolic extract-100mg/kg), CREE-200 (*Cuscuta reflexa* ethanolic extract-200mg/kg)

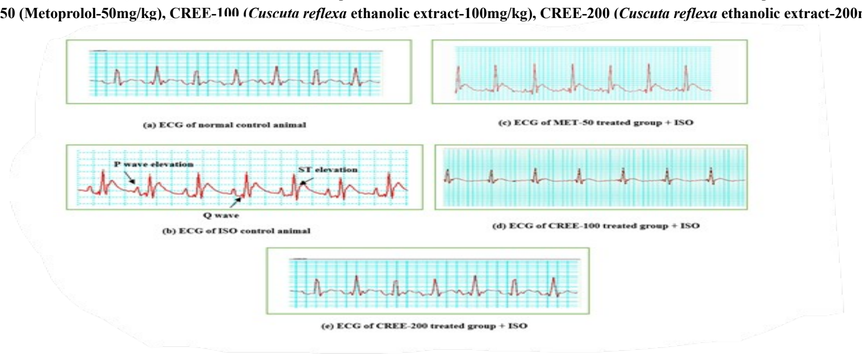

Fig. 3 ECG changes in different treatment groups

Effect on AST, ALT, CK-MB, CK-NAC and LD.

Table 2. Effects of CREE on serum ALT, AST, CK-MB, CK-NAC and LDH

Groups	AST(U/L)	ALT(U/L)	CK-NAC(U/L)	CK-MB(U/L)	LDH(U//L)
Normal control	7.97±1.66	18.86±1.39	16.65±1.01	46.30±3.73	107.10±2.80
ISO control (85mg/kg)	23.92±2.54***	34.4±0.85***	61.39±2.71***	135.123±3.41***	217.93±2.43***
MET (50mg/kg)	14.04±1.05$^{**\#\#\#}$	22.88±1.52$^{*\#\#\#}$	39.50±1.40$^{***\#\#\#}$	91.48±3.42$^{***\#\#\#}$	158.27±2.05$^{***\#\#\#}$
CREE (100mg/kg)	19.15±1.93$^{***\#}$	28.20±1.15$^{***\#\#}$	53.62±2.46$^{***\#\#}$	122.56±2.57$^{***\#\#}$	201.30±2.68$^{***\#\#}$
CREE (200mg/kg)	17.81±1.52$^{***\#\#}$	27.24±1.705$^{***\#\#}$	46.59±2.79$^{***\#\#}$	108.63±2.83$^{***\#\#}$	181.16±3.69$^{***\#\#}$

All values are mean ± SEM, n=6, $^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05 when compared to normal control; $^{\#\#\#}P$<0.001, $^{\#\#}P$<0.01, $^{\#}P$<0.05 compared to ISO control. ISO-85, MET-50, CREE-100, CREE-200

Effect on TNF-α, IL-6 and IL-10

The toxic control group treated with only ISO showed a significant increase in Pro-inflammatory markers TNF-α and IL-6 and a significant decrease in anti-inflammatory biomarkers compared to normal control. All the treatment groups, including MET-50, CREE-100 and CREE-200, restored the Pro-inflammatory and anti-inflammatory biomarkers. The CREE-200 treated group witnessed a much more accepted effect than the CREE-100 treated group.

Effect of CREE on SOD, Catalase, and GSH

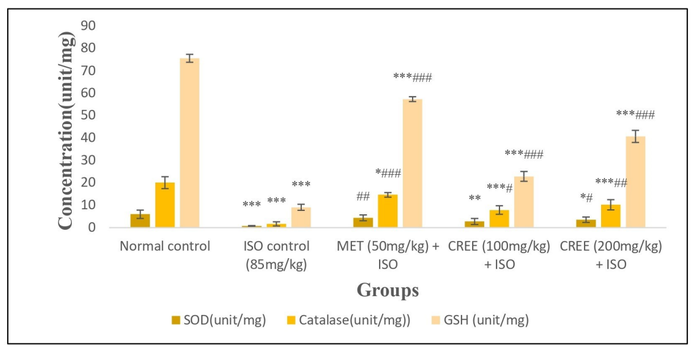

Fig. 4 Effect of CREE on SOD, CAT, and GSH

All values are mean ± SEM, n=6, $^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05 when compared to normal control; $^{\#\#\#}P$<0.001, $^{\#\#}P$<0.01, $^{\#}P$<0.05 compared to ISO control. ISO-85 (Isoproterenol-85mg/kg), MET-50 (Metoprolol-50mg/kg), CREE-100 , CREE-200.

Effect of CREE on histopathological studies

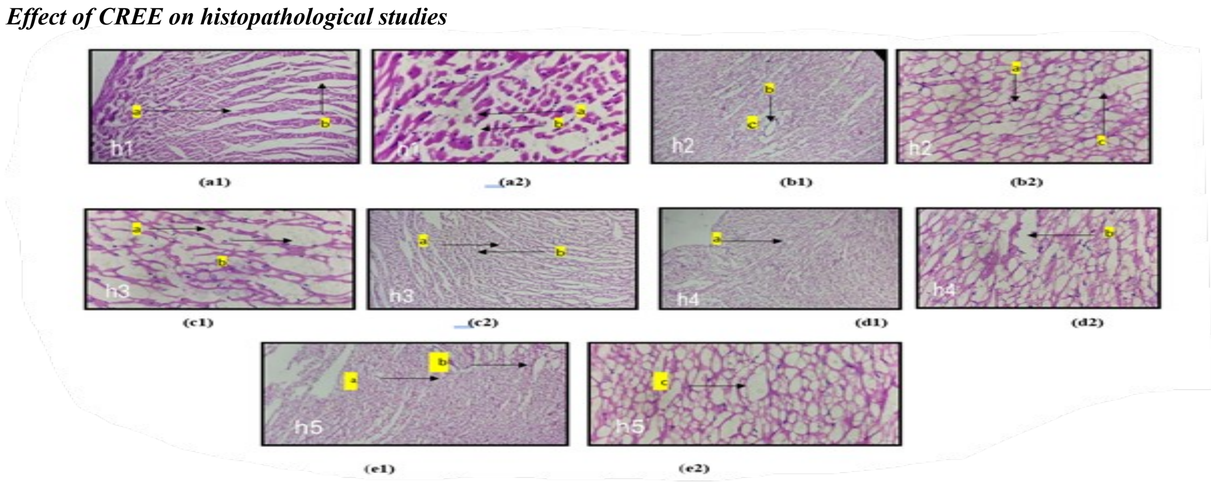

Fig. 5 Histopathological study of the myocardium

CONCLUSION

- *Cuscuta reflexa*, particularly its alkaloids, flavonoids, carbohydrates, saponins, glycosides, and tannins, holds promising potential for future applications in cardiac health.
- The ethanolic extract of *Cuscuta reflexa*, as studied, demonstrated a notable absence of ADR or ADE in the OECD TG-423 study, providing a reassuring safety profile for its use in *in-vivo* trials.
- The study result expresses the CREE's significant restoration on P wave, QT, and RR intervals. During the 21st day, serum parameters also showed progressive recovery under extract treatment.
- Our study also reported CREE's antioxidant properties against proinflammatory mediators and oxidative stresses. The histopathological investigations progressively provided insights into the recovery of reversible cellular injury.

FUTURE WORK

It is of utmost importance to profile secondary metabolites and their protein binding, absorption, cellular permeability, and molecular mechanism of *Cuscuta reflexa.* Furthermore, this plant is a promising candidate for treating clinical settings and will attract core researchers due to its diverse pharmacological properties.

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