



## Research Article

### CARDIOPROTECTIVE EFFECT OF *JATROPHA CURCAS* FRUIT EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED CARDIOTOXICITY IN RATS

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#### ABSTRACT

The present study was undertaken to explore the cardioprotective effect of *Jatropha curcas* fruit extracts against carbon tetrachloride induced cardiotoxicity in rats. Cardiotoxicity was induced by CCl<sub>4</sub> (3ml/kg body weight) in animals. Blood biochemical, hematological parameters and histopathological studies were carried to assess the cardioprotective effect. CCl<sub>4</sub> administration induced significant cardiotoxicity in rats, which was evident from enhanced levels of glucose, cholesterol, triglycerides and all hematological parameters. Pretreatment of silymarin (50mg/kg dose orally) significantly reversed carbon tetrachloride induced cardiotoxicity. From the obtained results it may be concluded that *Jatropha curcas* methanol extract (250mg/kg body weight) showed significant protective effect against CCl<sub>4</sub> induced cardiotoxicity in rats than *Jatropha curcas* aqueous extract (p<0.001) for most of the blood biochemical parameters, hematological parameters as well in attenuation of pathological changes in heart tissues.

**Key words:** Cardiotoxicity, cardio protective, carbon tetrachloride, silymarin, *Jatropha curcas*, *Curcas purgans*.

#### INTRODUCTION

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels and they include: coronary heart disease – disease of the blood vessels supplying the heart muscle; cerebrovascular disease – disease of the blood vessels supplying the brain; peripheral arterial disease – disease of blood vessels supplying the arms and legs; rheumatic heart disease – damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria; congenital heart disease – malformations of heart structure existing at birth; deep vein thrombosis and pulmonary embolism – blood clots in the leg veins, which can dislodge and move to the heart and lungs.

CVDs are the number one cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. Over three quarters of CVD deaths take place in low- and middle income countries. Out of the 16 million deaths under the age of 70 due to non-communicable diseases, 82% are in low and middle income countries and 37% are caused by CVDs. Most cardiovascular diseases can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies. People with cardiovascular disease or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia or already established disease) need early detection and management using counseling and medicines, as appropriate.

Clinical management of cardiovascular diseases is still a nightmare for the cardiologist. Thus far, vasodilators,  $\beta$  adrenergic blockers, antiarrhythmics, thrombolytics, etc. are the

mainstay of cardiac therapy. Analgesic agents like morphine have also been used. Most of the currently used therapeutic interventions provide only symptomatic relief.

Despite advances in western system of medicine and medical technology world over, its increasingly being realized that if we have to support the healthcare requirements of our ever increasing population, we will have resort to economical, yet effective alternatives and there cannot be a better alternative than the herbal drugs which have had a long history of safe usage in different parts of the world, including India.

The attention of the world is now being drawn more and more to herbs and herbal medicines as the synthetic drugs seem to have come up against a wall in the treatment of illness which is described as life style diseases<sup>1</sup>.

Silymarin is obtained from the *Silybum marianum* (milk thistle) an edible plant that has been used medicinally for the centuries as a herbal medicine. It is a mixture of mainly three flavonolignans, silybin, silidianin, and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, because of its antioxidant activity and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration<sup>2,3</sup>.

*Jatropha curcas* Linn. is a bush or small tree and belongs to Euphorbiaceae family. It is widely distributed in Mexico and Central America. The other name of the plant is *Curcas purgans*. Pharmacological reports revealed that it is having anti microbial<sup>4,5,6</sup>, anti-inflammatory<sup>7</sup>, antimetastatic<sup>8</sup>, antitumor<sup>8</sup>, coagulant and anti-coagulant (dose dependent), disinfectant, antiparasitic activity.

Carbon tetrachloride (CCl<sub>4</sub>), a clear, colorless and nonflammable synthetic liquid, is a renowned model compound for producing chemical tissue toxicity by creation of free radicals in liver, kidney, heart, lung, testis, brain and blood. It is bio transformed by hepatic microsomal cytochrome P450 to trichloromethyl-free radical (CCl<sub>3</sub> or CCl<sub>3</sub>OO), which in turn, instigate lipid peroxidation process. The most widely established means of CCl<sub>4</sub> induced cardiotoxicity is the creation of free radicals which is a rate limiting process in tissue peroxidative damage. The present study was conducted to examine the toxic upshots of CCl<sub>4</sub> plus to compare the beneficial effects of plant extracts on heart tissue of various experimental groups<sup>9</sup>.

## MATERIALS AND METHODS

### Animals

Wister rats of either sex, weighing around 200- 250 g. were employed in the present study. They were obtained from in house breed animals of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The rats were provided standard laboratory feed and water ad libitum. They were exposed to an alternate light and dark cycle of 12 h and had free access to food and water. The animals were acclimatized to the laboratory conditions for at least 5 days before the cardiotoxicity test. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh (Approval No. 09/IAEC/CIPS/2016-17; dt 05/04/2016) and care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forest, Environment, Climate Change, Government of India.

### Plant material

The plant material consists of dried powdered fruit of *Jatropha curcas* linn. belonging to the family Euphorbiaceae.

### Preparation of plant extract

Fresh fruits of *Jatropha curcas* linn. was collected from medicinal plant garden of Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur. India. The plant material was authenticated by a botanist and the specimen sample is deposited in the Pharmacognosy Division, Chalapathi Institute of Pharmaceutical Sciences. The shade dried and coarsely powdered fruits were extracted with solvents like methanol and water by hot percolation extraction (Soxhlation method) then the extracted solvents was filtered and filtrate was concentrated using a rotator evaporator. The concentrated plant extracts was used for the pharmacological activities.

### Drugs and reagents

Silymarin was purchased from Sigma Aldrich, Bangalore, India. CCl<sub>4</sub> and formalin was obtained from the Chalapathi Institute of Pharmaceutical Sciences. CCl<sub>4</sub> was administered intraperitoneally (i.p.) to induce cardiotoxicity in rats.

### Experimental groups

The efficacy of *Jatropha curcas* aqueous extract was compared *Jatropha curcas* methanolic extract by evaluating *in vivo* cardioprotective activity in rats against CCl<sub>4</sub> induced cardiotoxicity.

Four groups, each comprising of five wistar rats, were employed in the study.

Group I (Control group): Rats were administered 0.9% w/v normal saline, orally for 14 days.

Group II (CCl<sub>4</sub> - treated control group): Rats were administered CCl<sub>4</sub> (3 ml/kg, i.p.) on the day 14.

Group III (Silymarin + CCl<sub>4</sub>- treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14<sup>th</sup> day silymarin was administered 60 min prior the administration of CCl<sub>4</sub>.

Group IV (JCAE + CCl<sub>4</sub> -treated group): Rats were treated JCAE (250mg/kg body weight) for 14 days. On 14<sup>th</sup> day JCAE was administered 60 min before the administration of CCl<sub>4</sub>.

Group V (JCME + CCl<sub>4</sub> -treated group): Rats were treated JCME (250mg/kg body weight) for 14 days. On 14<sup>th</sup> day JCME was administered 60 min before the administration of CCl<sub>4</sub>.

### Parameters evaluated

- Blood biochemical parameters: glucose (mg/dl), calcium(mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), total protein (g/dl), sodium (mmol/lit).
- Hematological parameters: hemoglobin (mg/dl), total count (mm<sup>3</sup>), polymorphs(%), lymphocytes(%), esinophils(%), monocytes(%), packed cell volume (%),platelet count (lakhs/mm<sup>3</sup>), mean corpuscular hemoglobin count (%),red blood cells (million/mm<sup>3</sup>).
- Histopathological studies for heart tissues.

### Statistical analysis

The results are expressed as mean ± standard error of means (S.E.M.). The data of neurotoxicity results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test using graph pad prism version 6.0. A p-value <0.05 was considered to be statistically significant.

## RESULTS

Various pharmacological interventions employed in the present study did not show any significant mortality. Further, no significant difference was observed between the results obtained from rats of either sex.

### Effect on blood biochemical and hematological parameters

CCl<sub>4</sub> (3ml/kg i.p.) significantly increased levels of all blood biochemical parameters and hematological parameters except total proteins, total count and lymphocytes are decreased when compared to control group.

Silymarin (50 mg/kg, orally) pre-treated animals showed significant difference in blood glucose, triglycerides, total count, total proteins, hemoglobin differential count and in PCV which evident from Table 1 & 2.

JCAE (250mg/kg body weight) pre-treated animals showed significant decreased levels of all blood biochemical parameters and hematological parameters except glucose, sodium, total count, differential count, platelet count and red blood cells.

JCME (250mg/kg body weight) pre-treated animals showed significant decreased levels of all blood biochemical parameters and hematological parameters except glucose, sodium, hemoglobin, total count, lymphocytes, mean corpuscular hemoglobin count and red blood cells which is evident from Table 1 & 2.

Histopathological studies of CCl<sub>4</sub> induced cardiotoxicity group showed significant damage in heart tissue when compared with control group. The CCl<sub>4</sub> treated group showed congested vessels and necrosis where as in control group no significant abnormalities was detected The histopathological slides of silymarin pre-treatment group showed significantly attenuated

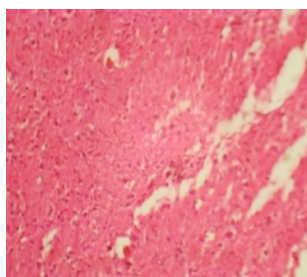
congested non-specific inflammation when compared with CCl<sub>4</sub> group. The histopathological slides of JCAE treated group showed chronic inflammation where as JCME group showed significant recovery of heart tissue after CCl<sub>4</sub> challenge similar to silymarin treatment group as shown in Figure 1.

**Table 1: Blood biochemical parameters of various treatment groups**

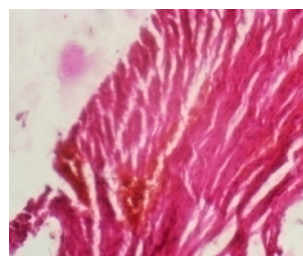
S.No.	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Glucose (mg/dl)	22.4±2.99	60.2±2.98	49.6±1.15	67.8±5.28	71±5.29
2	Calcium(mg/dl)	12.2±0.31	12.1±0.56	13.78±0.18	8.61±0.19	11.2±0.49
3	Cholesterol (mg/dl)	41.4±1.07	81.8±6.73	70.6±3.07	51±3.37	71±2.93
4	Triglycerides (mg/dl)	52.6±3.7	110±10.57	99.4±6.75	65.2±3.12	100.8±3.26
5	Total Protein (g/dl)	9.22±0.36	8.94±0.62	6.9±0.14	5.54±0.37	5.5±0.14
6	Sodium (mmol/lit)	135.4±1.5	116.6±5.44	134.8±1.88	138.8±2.35	135.6±1.312

**Table 2: Hematological parameters of various treatment groups**

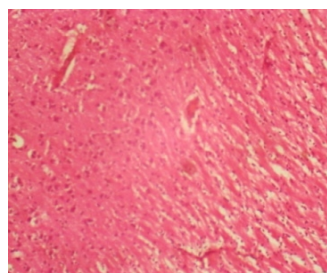
S.No.	Parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
1	Haemoglobin (mg/dl)	13.56±0.21	16.4±0.39	13.41±0.76	13.36±0.24	16.80±0.73
2	Total Count(mm <sup>3</sup> )	3800± 291.54	2440± 156.84	1540± 166.13	3580±251.79	4360±136.38
3	Differential Count (%)					
	Polymorphs	30±4.29	36.6±1.28	31.6±2.16	34±1.37	34±1.78
	Lymphocytes	62.8±4.72	55±2.05	60.6±3.39	64.6±2.03	60±0.83
	Esinophils	2.2±0.86	4.4±1.02	2.6±0.51	2±0.70	3.2±0.86
	Monocytes	2.4±0.81	5±0.71	2.4±0.81	3±0.83	3.2±0.37
4	Packed Cell Volume (%)	42.4±0.92	50.2±0.86	38.2±2.31	35.2±2.56	48.8±1.15
5	Mean Corpuscular Haemoglobin Count (%)	31±0.32	32.8±0.37	34.4±0.51	32.8±0.37	33.4±0.50
6	Platelet Count (lakhs/mm <sup>3</sup> )	2.94±0.22	4.86±0.57	3.44±0.65	6.9±0.10	5.04±0.36
7	Red Blood Cells (million/mm <sup>3</sup> )	7.3±0.26	8.87±0.17	7.22±0.44	6.81±0.35	7.78±0.38



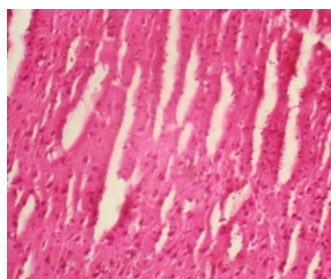
A) Control group



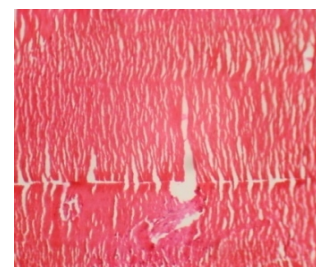
B) Positive control group



C) Silymarin treatment group



D) Aqueous extract treatment group



E) Methanolic extract treatment group

**Figure 1: Histology slides of the isolated heart of various treatment groups showing haematoxylin and eosin stained cells**

**DISCUSSION**

Carbon tetrachloride induced cardiotoxicity employed in the present study is one of the most widely accepted models to evaluate cardioprotective activity in laboratory animals<sup>10,11</sup>. In the present study, CCl<sub>4</sub> treatment group showed a significant increase in observed blood biochemical parameters, hematological parameters except calcium (p<0.0001), total

proteins (p<0.0001), sodium (p<0.002), total count, lymphocytes when compared with control group.

Pretreatment with silymarin attenuated CCl<sub>4</sub> induced cardiotoxicity when compared with positive control group which is evident form significant decrease in blood biochemical parameters, hematological parameters except calcium (p<0.05), sodium (p<0.001), lymphocytes and mean corpuscular hemoglobin count.

Silymarin treatment group showed decrease in blood biochemical parameters, hematological parameters except glucose ( $p<0.0001$ ), calcium, cholesterol ( $p<0.0001$ ), triglycerides, polymorphs, eosinophils, mean corpuscular hemoglobin count ( $p<0.0001$ ), platelet count when compared with control group. Monocyte count was found to be similar between silymarin and control group.

Pretreatment with JCAE showed decreased in blood biochemical parameters, hematological parameters except glucose ( $p<0.05$ ), sodium, total count ( $p<0.0001$ ), polymorphs, lymphocytes, monocytes, platelet count ( $p<0.0001$ ) when compared with silymarin group.

Pretreatment with JCAE showed decrease in blood biochemical parameters, hematological parameters except glucose, sodium ( $p<0.0001$ ), total count ( $p<0.001$ ), lymphocytes, platelet count ( $p<0.05$ ), when compared with  $CCl_4$  group. Mean corpuscular hemoglobin count was found to be similar with  $CCl_4$  group.

Pretreatment with JCAE showed increase in blood biochemical parameters, hematological parameters except calcium ( $p<0.0001$ ), total proteins ( $p<0.001$ ), hemoglobin, total count, eosinophils, packed cell volume, red blood cells when compared with control group.

Pretreatment with JCME showed increased in observed blood biochemical parameters, hematological parameters except sodium, lymphocytes, platelet count ( $p<0.05$ ) when compared with JCAE group. Polymorph count was found to be similar with JCAE group.

Pretreatment with JCME showed increase in blood biochemical parameters, hematological parameters except calcium ( $p<0.001$ ), total proteins and mean corpuscular hemoglobin count when compared with silymarin group. Lymphocyte number was found to be similar with silymarin group.

Pretreatment with JCME showed decrease in blood biochemical parameters, hematological parameters except glucose, sodium ( $p<0.001$ ), hemoglobin, total count ( $p<0.0001$ ), lymphocytes, mean corpuscular hemoglobin count, platelet count when compared with  $CCl_4$  group.

Pretreatment with JCME showed increase in blood biochemical parameters, hematological parameters except calcium, total proteins ( $p<0.001$ ) and lymphocytes when compared with control group. Sodium levels were similar with control group.

The histopathological slides of JCAE treated group showed chronic inflammation where as JCME group showed significant recovery of heart tissue after  $CCl_4$  challenge similar to silymarin treatment group.

## CONCLUSION

From the obtained results it may be concluded that *Jatropha curcas* methanol extract showed significant effect against  $CCl_4$  induced cardiotoxicity in rats than *Jatropha curcas* aqueous extract for most of the blood biochemical parameters, hematological parameters ( $p<0.001$ ) as well in attenuation of pathological changes in heart tissues.

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