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Research Article

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Evaluation of iron chelating potential of methanolic extract of seeds of *Celastrus paniculatus* on iron intoxicated rats

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ABSTRACT

The seeds of Celastruspaniculatuswere collected and pulverized to fine powder and the powder was extracted by Soxhlet (continuous Hot Extraction) apparatus using methanol as solvent. In-vitro studies were carried out using various reagents like Ferrous Sulphate, Potassium thiocynate, Ferrozine, Desferrioxamine, purified water and methanolic extract of crude drug. The % iron-inhibition of standard and test drugs were measured. Animal studies were done using Iron intoxicated rats and Albino Wistar rats were subjected for iron dextrose injection (i. p.) six times in 21 days to all the groups (except normal control) and after treatment duration, Serum iron level, SGOT, SGPT, CKMB & Creatinine levels were measured. The iron chelating activity of Celastruspaniculatus was confirmed by in-vitro evaluation. There was significant decrease in serum iron levels in test group as compared to disease control group animals. The organo-protective effects on liver, heart and kidney was confirmed by reduction in various bio-markers like SGPT, SGOT, Creatinine and CKMB levels as compared to iron overloaded rats. The results of the study has shown that the methanolic extract of Celastruspaniculatus have significant iron chelating activity. It also has beneficial effects on hematological parameters and organo-protective effects were observed during study. So it can be useful in the management of iron overload disorders like thalassemia and hemochromatosis like conditions.

Keywords *Celastruspaniculatus*, iron-chelation, phenolic content, iron dextran induced iron-intoxication.

INTRODUCTION

Iron is an absolute requirement in the biological processes of many organisms, the primary role of iron in the body is to provide a binding site for oxygen in the heme moiety of hemoglobin. Iron also plays an important role in the enzymatic transfer of electrons in oxidation and reduction reactions that are performed by cytochromes and many other enzymes[1].

The iron also has the potential to do great damage to biological systems if it is not attenuated by iron-binding proteins. If iron-binding capacity is reduced, free iron is capable of promoting the formation of reactive oxygen species and peroxide free radicals which may damage cellular components, such as nucleic acids, cellular membrane proteins, DNA, and lipids and ultimately kill the cell.

Iron overload disorders are the diseases caused by the excessive accumulation of iron in the body. The iron overload results when the amount of circulating iron is higher than the amount of transferrin available to bind it in the blood[2].

Iron overload may be genetic or acquired by receiving numerous blood transfusions or getting iron infusions. Excess iron in the vital organs, increases the risk of liver damage (cirrhosis, cancer), heart damage, diabetes mellitus[3,4], osteoarthritis, osteoporosis, hypothyroidism[5], hypoparathyroidism[6], impaired growth[7] and numerous symptoms and in some cases premature death.

For the treatment of iron overload many synthetic agents are used such as Desferrioxamine and Deferiprone, but they are showing serious side effects. These drugs are having certain limitations including parenteral administration, high cost and they are not used in the pregnant women[8].

Drawbacks of synthetic agents[8]
Numerous and serious ADRs
Poor oral bioavailability
Short plasma half-life

Compared to synthetic drugs, the herbal preparations are quite less toxic with very less side effects. The poor oral bioavailability, short plasma half-life and severe side effects of currently available chelators are still not optimal[8]. So the researchers and scientists move towards research on the herbal alternates that are more safe and efficacious.

Thus objective of present study is to evaluate new herbal drug having iron chelating potential and beneficial effects on other iron overload complications with least side effects.

Due to the need of a new safe and efficacious iron chelating molecule, we resolved to investigate beneficial effects of *Celastruspaniculatus*seeds on iron overload disorders.

The plant *Celastruspaniculatus* is a perennial shrub which belongs to family Celastraceae. It is formerly known as *Malkangani*, is the climbing shrub grows all over India[9]. It is also called as *jyotishmati* or *intellect tree*, because it is traditionally used to improve memory and CNS disorders. Mainly the seeds and oil obtained from seeds are used in the formulations. Since many years, according to the Ayurvedic literatures, it is used in CNS disorders and memory enhancer drug[9].

It contains high amount of flavonoids and phenolic compounds, which may show the metal chelation properties. The phenolic compounds and the flavonoids chelate iron in the body and reduce the iron overload. Because of this content it may be useful in metal intoxication, especially iron overload conditions.

Celastruspaniculatus is very beneficial as Nootropic[10], Anti-inflammatory[11], Analgesic[11], Anti-oxidant[12], Hypolipidemic[13], Anti-arthritic[14], Anxiolytic[15], Wound healing[16], Ileum muscle relaxant[17]. All these activities are proved and reported in the various literature.

There is no significant or severe adverse effects yet reported for *Celastruspaniculatus*. This is the main advantage and also a rational for choosing this drug and to evaluate its iron chelating potential and beneficial effects in iron overload disorders.

EXPERIMENTAL SECTION

1. Collection and Authentication

The seeds of *Celastruspaniulatus*was collected from local area of Rajkot region, Gujarat. The crude drug was authenticated by Ms. TruptiMarkana, Botany department, School of Science, RK University, Rajkot, Gujarat.

2. Extraction

The crude drug was collected, dried and pulverized to fine powder. Powder was filled inside the thimble and extracted by Soxhelet (Continuous Hot Extraction) apparatus using methanol as the solvent.

3. Quantitative measurement of Phenolic Content

Reagents – distilled water, Gallic acid, Sodium carbonate, Folin-coicalteu (FC)
Requirements - Glass beakers, Measuring cylinders, Graduated pipettes, Micropipette, UV-Visible Spectrophotometer

Fig. 1 : UV-Visible Spectrophotometer

Procedure – 50 µl of gallic acid or blank (distilled water) is taken in the test tube and 1.58 ml of distilled water is added into it.100 microliter FC reagent is added in the mixture and it is allowed to incubate for 8 minutes. Afterwards 300 µl of sodium carbonate solution (2 gm in 8 ml) is added into the sample and again incubated for 1 hour at room temp.

The final volume of 2 ml was made using distilled water. The solution is filled into the cuvettes and absorbance is measured at 765 nm in UV-Visible spectrophotometer.

Calculations – The conc. of phenolic substance was measured by using following equation:
$C_u / C_s = A_u / A_s$
C_u = Concentration of unknown
C_s = Concentration of standard
A_u = Absorbance of unknown
A_s = Absorbance of standard
4. *In-vitro* studies:
Model – 1

Reagents – Ferrous Sulphate, Ferrozine, Desferrioxamine, Purified water, methanolic extract of crude drug

Requirements–Glass beakers, Measuring cylinders, Graduated pipettes, Micropipette, UV-Visible spectrophotometer

Procedure:– 100 microliter of Ferrous Sulphate solution was taken into the clean and dry cuvette. For the control group, 2 ml of distilled water was added into the solution. To this solution, 0.4 ml of Ferrozine solution was added and mixed well. The mixture was allowed to stand for 5 min to complete the reaction. After 5 min the cuvette was placed inside the spectrometer and the absorbance was taken at 562 nm using distilled water as a reference.

The above procedure was repeated, in the case of test group using 2 ml of methanolic extract of crude drug in DMSO instead of distilled water and the reference used was distilled water along with 2 ml of extract in DMSO, to balance the opacity in both the cuvettes.

Similarly, in case of standard groupthe 2 ml of Desferroxamine solution was taken in place of distilled water and absorbance was taken using distilled water as a reference.

Calculations:– The % iron-inhibition of standard and test drugs were measured by using following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

Where,
A_0 = Absorbance of control group
A_s = Absorbance of test drug (For test group)
A_s = Absorbance of std. drug (For std. group)

MODEL – 2
Reagents – Ferrous Sulphate, Potassium thiocynate, Desfrrioxamine, Purified water, methanolic extract of crude drug.
Requirements–Glass beakers, Measuring cylinders, Graduated pipettes, Micropipette, UV-Visible spectrophotometer

Procedure:– 100 µl of Ferrous Sulphate solution was taken into the clean and dry cuvette. For the control group2 ml of distilled water was added into the solution. To this solution, 0.4 ml of Potassium thiocynate solution was added and mixed well. The mixture was allowed to stand for 5 min to complete the reaction. After 5 min the cuvette was placed inside the spectrometer and the absorbance was taken at 480 nm using distilled water as a reference.

The above procedure was repeated, in the case of test group using 2 ml of methanolic extract of crude drug in DMSO instead of distilled water and the reference used was distilled water along with 2 ml of extract in DMSO, to balance the opacity in both the cuvettes.

Similarly, in case of standard groupthe 2 ml of Desferrioxamine solution was taken in place of distilled water and absorbance was taken using distilled water as a reference.

Calculations:– The % iron-inhibition of standard and test drugs were measured by using following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s \times 100}{A_0}$$

Where,
A_0 = Absorbance of control group
A_s = Absorbance of test drug (For test group)
A_s = Absorbance of std. drug (For std. group)
5. Animal model (Iron Dextrose induced Iron-intoxicated Rats Model)

Selection of animals
Healthy Wistar rats weighing 200-250 gm, guinea pig weighing 350-500 gm and mice weighing 25-30 gm were used for the study. The animals were housed in a group of 3 rats per cage under well –controlled conditions of temperature (22 ± 2^0 C), humidity ($55 \pm 5\%$) and 12h/12h light-dark cycle. Animals had received standard pellet diet (Pranav agro, Baroda) and drinking water adlibitum. Animals were divided into different groups for different models. The protocol of the experiment was approved by Institutional Animal Ethical Committee(IAEC) as per guidance of the Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No:- RKCP/COL/RP/15/62).

Model used – Iron intoxicated rats
Animals used – Albino Wistar rats of either sex
No. of animals used – 6/group
No. of groups – 4

Parameters measured – 1.Serum Iron level
2. Serum SGPT level
3. Serum SGOT level
4. Serum Creatinine level

During the study of 21 days, the iron overload was produced by iron dextrose solution which was given at 3 days interval for 6 times by i.p. route, except normal control group. The test group animals were given 12.5 mg/ 100 gm dose of crude drug extract by p.o. route per day. The standard group animals were given Desferrioxamine 40 mg/ kg by p.o. route per day.

After 21 days the blood samples were collected from animals by retro-orbital plexus method.
The collected blood samples were centrifuged at -5˚ C at 2500 rpm using REMI
Cooling centrifuge equipment and serum was separated.

Fig. 2: REMI Cooling Centrifuge

a) Serum Iron level

Reagents - Ferrozine, Purified water, Serum samples from different groups of animals

Requirements:- Glass beakers, Measuring cylinders, Graduated pipettes, Micropipette, UV-Visible Spectrophotometer

Procedure:- 100 µl of serum sample was taken into the clean and dry cuvette. For the normal control group, 2 ml of distilled water was added into the solution. To this solution, 0.4 ml of Ferrozine solution was added and mixed well. The mixture was allowed to stand for 5 min to complete the reaction. After 5 min the cuvette was placed inside the spectrometer and the absorbance was taken at 562 nm using distilled water as a reference.

The above procedure was repeated, in the case of Disease control group, Standard groupand Test group using respective serum samples.

Calculations:- The % iron-inhibition of standard and test drugs were measured by using following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

Where,

A_0 = Absorbance of control group

A_s = Absorbance of test drug (For test group)

A_s = Absorbance of std. drug (For std. group)

6. Statistical Analysis

Statistical analysis was performed by using ANOVA technique to determine the variance in the data within the groups and between the groups.

RESULTS

1. Morphological Studies of *Celastruspaniculatus* Seeds

Fig. 3: Morphology of Seeds of *Celastruspanicuatus*

Table 1: Morphological Parameters of Seeds of *Celastruspanicuatus*

Colour	Yellowish Brown
Size	0.5 cm
Shape	Ovate to Oblonged
Surface	Smooth

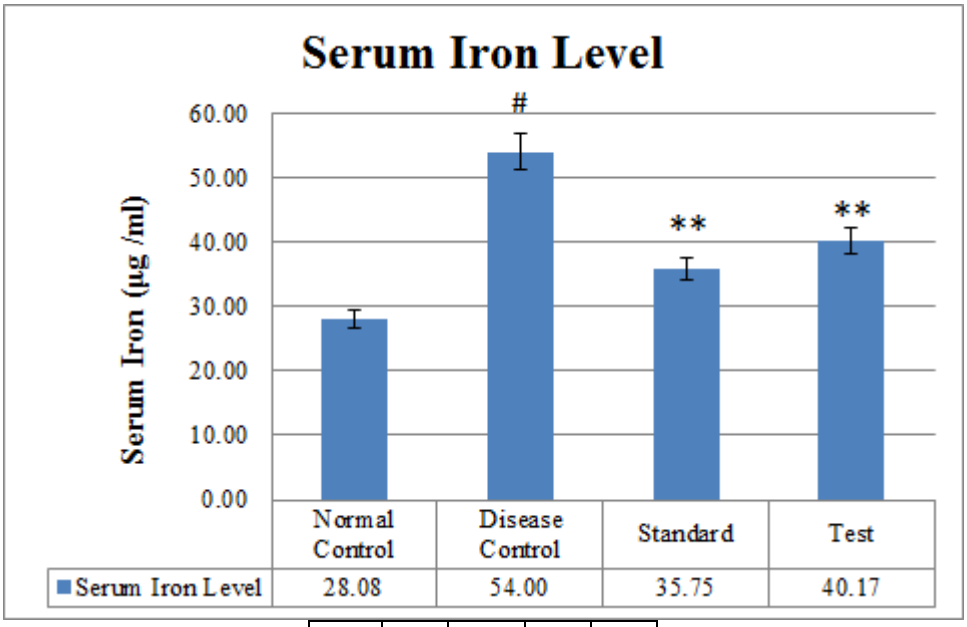
2. Morphological Studies of MECP (Methanolic extract of seeds of *Celastruspaniculatus*)

Table 2: Morphological parameters of MECP (Methanolic Extract of Seeds of *Celastruspanicuatus*)

Extract	MECP
% w/w Yield	43.33%
Colour	Yellowish Brown
Consistancy	Semi-solid

3. % Phenolic content present in MECP = 30.98 %
4. Animal studies

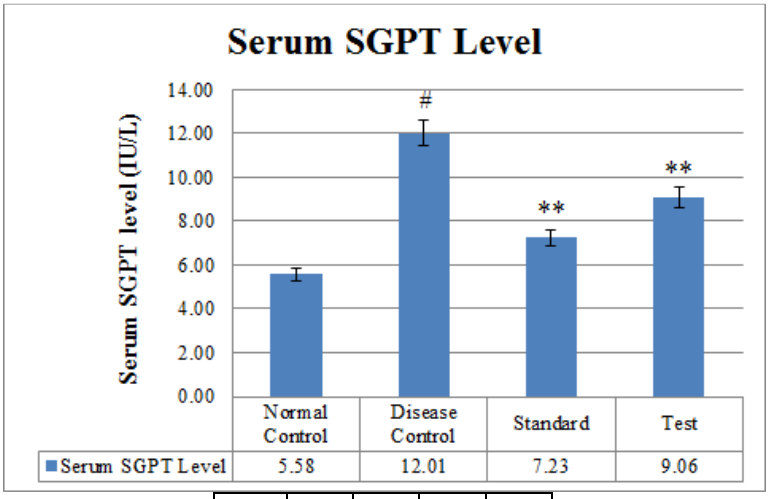
a) Serum Iron level



SD	4.45	10.65	5.80	9.11
SEM	1.82	4.35	2.37	3.72

P value = 0.000143223
(P value < 0.001)
Standard – Desferrioxamine
Test – MECP
= significant difference between disease control group and normal control
** = significant difference between test group/ standard group and normal control group
P value is less than 0.001 so it shows highly significant difference between the serum iron level of disease control group and that of the test group as well as that of the standard group.
The result shows that there is also a significant difference in iron levels of normal control group and disease control group.

b) Serum SGPT level

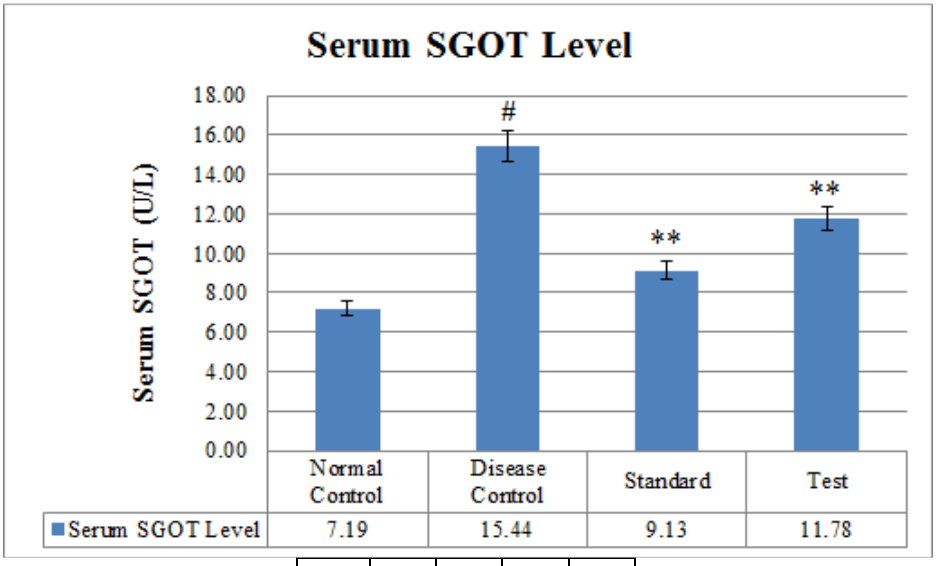


SD	2.48	2.62	2.43	1.94
SEM	1.01	1.07	0.99	0.79

P value = 0.001030926
(P value < 0.001)
Standard – Desferrioxamine
Test – MECP
= significant difference between disease control group and normal control
** = significant difference between test group/ standard group and normal control group
P value is less than 0.001 so it shows highly significant difference between the serum SGPT level of disease control group and that of the test group as well as that of the standard group.

The graph shows that there is also a significant difference in SGPT levels of normal control group and disease control group.

c) Serum SGOT level
d)



SD	2.26	1.61	1.75	1.31
SEM	0.92	0.66	0.72	0.53

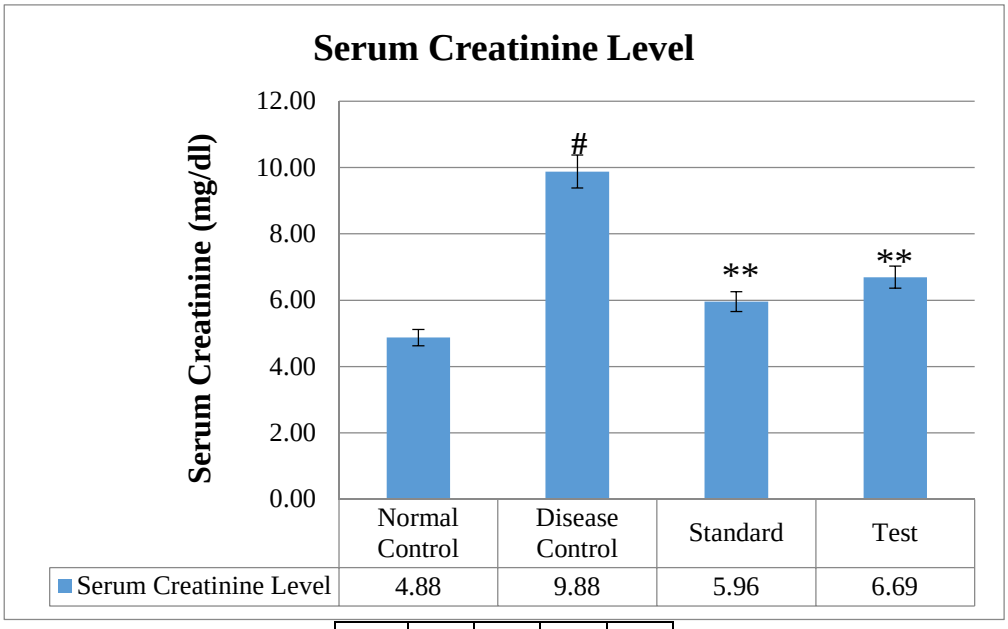
P value = 0.000000676491
(P value < 0.001)

Standard – Desferrioxamine
Test – MECP
= significant difference between disease control group and normal control
** = significant difference between test group/ standard group and normal control group

P value is less than 0.001 so it shows highly significant difference between the serum SGOT level of disease control group and that of the test group as well as that of the standard group.

The comparison graph shows that there is also a significant difference in SGOT levels of normal control group and disease control group.

e) Serum Creatinine level



SD	1.03	1.79	1.78	2.09
SEM	0.42	0.73	0.73	0.85

P value = 0.000436973
(P value < 0.001)
Standard – Desferrioxamine
Test – MECP
= significant difference between disease control group and normal control
** = significant difference between test group/ standard group and normal control group
P value is less than 0.001 so it shows highly significant difference between the serum Creatinine level of disease control group and that of the test group as well as that of the standard group.

The result shows that there is also a significant difference in Creatinine levels of normal control group and disease control group.

Table 3: Combined results of all the parameters measured

Group	Serum Iron	Serum SGPT	Serum SGOT	Serum Creatinine
Normal control	28.08 ± 1.82	5.58 ± 2.28	7.19 ± 2.94	4.88 ± 0.42
Disease control	54 ± 4.35	12.01 ± 5.90	15.44 ± 6.30	9.88 ± 0.73
Standard	35.75 ± 2.37	7.23 ± 2.95	9.13 ± 3.73	5.96 ± 0.73
Test	40.17 ± 3.72	9.06 ± 3.70	11.78 ± 4.81	6.69 ± 0.85
F	9.4175666	8.051849	24.450338	9.417566557
df	23 (3,20)	23 (3,20)	23 (3,20)	23 (3,20)
P	0.000437	0.0010309	6.76E-07	0.000436973

DISCUSSION

The in-vitro models [(1) % inhibition of Ferrozine (2) % inhibition of Potassium thiocynate] the test drug shows the highly significant iron chelation, so it is tested in the animal models and it also shows the similar positive results. In the iron-intoxicated rats model, the methanolic extract of *Celastrus paniculatus* seeds shows significant decrease in serum iron levels. It also significantly decreased the Liver and Kidney function biomarkers such as serum SGPT level, serum SGOT levels and serum Creatinine levels. From this we can say that the test drug minimizes the toxicity of iron overload and also reduces the liver function damage.

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