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Evaluation of Beneficial Effects of *Medicago Sativa* (Alfalfa) In Iron-Overload Conditions

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Abstract: In medicine, iron overload indicates accumulation of iron in body from any cause. The most important causes are thalassemia, hereditary haemochromatosis (HHC) and transfusional iron overload, which can result from repeated blood transfusion. Aim of present study is to investigate *in-vivo* iron chelating potential and beneficial effects of *Medicago sativa* in iron overload and its complications. Iron chelating and organo protective activity of methanolic (250 mg/kg) and water (500 mg/kg) extracts of *Medicago sativa* along with standard drug desferoxamine were assessed against iron-dextran induced iron overload models in Wistar rats which results in condition of chronic iron overload found in thalassemia patients. At the end of 15 and 30 days of in-vivo trial, serum iron and ferritin , urine and fecal iron levels including complications on vital organ by histopathological study and test for biomarkers were (SGPT, SGOT, Serum creatinine, creatine kinase) were estimated to measure organo protective effects. There were significant decreased in serum iron, ferritin were observed compared to iron overloaded rats. These beneficial effects were observed because methanol and water extracts of *M. sativa* increased excretion of iron in urine and fecal due to iron chelation property of extract. Organo-protective effects on liver, heart and kidney of both extracts of *M. sativa* in iron overloaded rats were confirmed by histopathological study and reduction in various markers like SGPT, SGOT, creatinine, creatinine kinase levels. Data of our study confirmed that methanol and water extracts of *Medicago sativa* have significant iron chelating activity and organo-protective effects in iron overload conditions which is very beneficial in management of iron overload disorders like thalassemia, haemochromatosis like conditions.

KeywordsL: Iron, *Medicago sativa*, desferoxamine, Chelating activity, organo-protective

INTRODUCTION

In medicine, iron overload disorders are diseases caused by the accumulation of iron in the body. Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it. The type of acute toxicity from iron ingestion causes severe mucosal damage in gastrointestinal tract, among other problems. Iron overload is one of the major causes of morbidity in all patients with severe forms of thalassemia, regardless of whether they are regularly transfused. A variety of other iron overload diseases are present. These are thalassemia, sideroblastic anemia, abnormal red cell production (dyserythropoiesis), iron overload secondary to IV therapy, chronic liver disease secondary to alcohol, porphyria cutanea tarda[1].Iron overload can be inherited (genetic) or acquired by receiving numerous blood transfusions, getting iron shots or injections, Oral Rehydrated Solutions consuming high levels of supplemental iron. Thalassemia is one of the genetic disorders which results in iron overload in body.

Excess iron in vital organs, even in mild cases of iron overload, increases the risk for liver disease (cirrhosis, cancer), heart attack or heart failure, diabetes mellitus[2,3] osteoarthritis, osteoporosis, metabolic syndrome[4], hypothyroidism[5], hypoparathyroidism[6], hypogonadism[6,7], impaired growth[8], numerous symptoms and in some cases premature death. Iron mismanagement resulting in overload can accelerate such neurodegenerative diseases as Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy and multiple sclerosis[9, 10]. Iron overload is major problem found in thalassemia major patients.

Synthetic agents like desferrioxamine and deferiprone used for the treatment of iron overload in thalassemia are accompanied by serious side effects and certain limitations including need for Parenteral administration, arthralgia, nausea, gastrointestinal symptoms, fluctuating liver enzyme levels, leucopenia, agranulocytosis and zinc deficiency and obviously the heavy cost. In addition, they are not suitable for use during pregnancy[11-13]. Compared to synthetic drugs, herbal preparations are frequently less toxic with fewer side effects. Therefore the search for more effective and safer treatment of thalassemia and other iron overload conditions are new area of research.

The poor oral bioavailability, short plasma half-life and severe side effects of available chelators are still not optimal[14-16]. Within this context and taking in consideration the relative paucity of iron chelating agents it is not surprising that clinical scientists put a great effort towards finding any potentially useful sources in order to obtain the maximum possible benefit with the least possible harm[17,18]. Thus objective of present study is to evaluate new herbal drug having potent iron chelating potential and beneficial effect in other iron overload complications and with least adverse effects.

Looking at the dire need of a new safe and economical iron chelating molecule, we resolved to investigate beneficial effect of *Medicago sativa* on iron overload in thalassemia. The plant *Medicago sativa* Linn. (Alfalfa) belongs to family Fabaceae is locally known as 'buffalo herb of Lucern', in gujarati its known as 'Rajko' or 'Gadab'[19].Evidence have also found that Phenols and flavanoids have **iron chelation activity** and Medicago sativa contains phenol and flavanoid and have antioxidant activity[20]. It chelates iron in the body and reduces iron overload and thus thalassemia. *M. sativa* contains other chemical constituents like cystine, phytosterol, saponin, coumarins, amino acids, Vit. A, K, C, E, Zn, Selenium, minerals. *Medicago sativa* is taken for a wide range of conditions, including allergies, morning sickness, arthritis, digestion, gout, anemia, rheumatism, blood clotting

agent, blood purifier, tooth decay, bone strengthener and urinary problems [21]. *Medicago sativa* is useful for breaking down toxins in the blood system[22].*Medicago sativa* are very beneficial as neuroprotective, hypocholesterolemic, antioxidant, antiulcer, antimicrobial, hypolipidemic, estrogenic, and in the treatment of atherosclerosis, heart disease, stroke, cancer, diabetes and menopausal symptoms in women which are reported in various literature[23].Objective of my work is to find out mode of action and beneficial effect of various extracts of *Medicago sativa* in iron-dextran induce iron overload in rats. Also access the organo protective effect of *Medicago sativa* through its iron chelating activity.

EXPERIMENTAL

Collection and Authentification: The arial part of *Medicago sativa* was collected from local area of Rajkot region, Rajkot, Gujarat. The crude drug was authentified by Prof. Vishal Muliya, botanical department, Christ College, Rajkot, Gujarat, India. Herbarium authentified sample of *M. sativa was* prepared according to standard procedure (Herbarium No. SOP/COG/1/2013).

Preparations of Various Extract: The crude drugs was collected, dried and pulverized to fine powder. Powder was macerated first with cold water and then with methanol. The extracts were filtered and concentrated under reduced pressure and adjusted with known volume. Resulting extracts were transferred into evaporating disc and left overnight in a stream of air to produced dry residue, which was further used for evaluation process[24].

Selection of Animals: Either sex Wistar rats of weighing 200-220 g were used for the study. The animals were collected from Animal House, Department of Pharmacology, School of Pharmacy, RK University Rajkot, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2˚C and relative humidity of 30 – 70 %. A light and dark cycle was follow. All animals were fed on standard balance diet and provided with water *ad labium.*

All the experimental procedures and protocols used in study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and care of laboratory animals were taken as per the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India (Protocol No. RKCP/COL/RP/13/39).

Induction of Iron overload: Either sex of Wistar rats of initially weighing 200-220 gm were used for present study. The rats were given six Intraperitoneal injections of iron-dextran (12.5 mg/l00 g body wt.) evenly distributed over a 30 days of period which results in condition of chronic iron overload[25,26]. Control rats were injected with an equal volume of dextrose at the same time. The experimental animals were divided into five groups, (n=6).

- Group 1(n=6):Normal control received dextrose solution (NC).
- Group 2(n=6): Disease control treated with Iron Dextran (DC) (12.5mg/l00g body wt.) Intraperitoneal injections for 30 days.
- Group 3(n=6): Disease control treated with Desferoxamine(DCD) (40 mg/kg, p.o., per day).
- Group 4 (n=6):Disease control treated with aqueous extract of *M. sativa* (DCWM) (500mg/kg p.o. per day).
- Group 5(n=6):Disease control treated with methanolic extract of *M. sativa* (DCMM)(250mg/kg p.o. per day).

Blood samples, urine samples and fecal samples were collected on 15 and 30 days under fasting conditions, from retro orbital plexuses using light ether anesthesia, in clean dry centrifuge tubes. Histopathology of heart, liver and kidney were performed at the end of 30 days to study iron overload complications. Collected blood samples were allowed to clot for 30 min at room temperature and serum was separated by cooling centrifugation at 5000 rpm for 20 min and stored at –20˚ C until the analysis was carried out.

Estimated Parameters

Serum Iron: Sampling, reagent delivery, mixing, processing and printing of results were automatically performed by RA-50 fully automated Analyzer**.**

Serum Ferritin[25]: Sampling, reagent delivery, mixing, processing and printing of results were automatically performed by VIDAS- fully automated Analyzer.

Urine and Fecal Iron Level: All urine samples were centrifuged at 14000 rpm for 5 min to remove mucus and epithelial cells and digested samples of fecal[27] were directly injected into fully automated Analyzer- RA-50 Analyzer for measurement of Iron level.

Biomarkers measurement: Various markers like SGPT, SGOT, serum creatinine and creatine kinase (CKMB) were measured using biomarker measurement kit.

Histopathological Study: Histopathological study of major organs included heart, liver and kidney were carried out to study protective effects of different extracts of *M. sativa* on iron overload induce complications on animals.

Statistical Analysis: To checking 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.

RESULTS AND DISCUSSION

In-vivo* Evaluation of Iron Chelating Activity in Various Extracts of *Medicago sativa: Intraperitoneal injections of iron-dextran (12.5 mg/l00 g body wt.) evenly distributed over a 30 days period that results in condition of chronic iron overload (serum iron – 477.50 ± 34.66 µg/dl) which is found as same as iron overload disorder condition. Control rats were injected with an equal volume of dextran at the same time showed normal level of iron (serum iron – 199.33 ± 19.95 µg/dl) in rats. All the studies were carried out for a period of 30 days. During the blood sample, urine sample and facial sample were collected on 15 and 30 days under fasting conditions.

Serum iron and serum ferritin levels: Intraperitoneal injections of iron-dextran (12.5 mg/l00 g body wt.) evenly distributed over a 30 days period on wistar rats resulted in condition of chronic iron overload (serum iron – 477.50 ± 34.67µg/dl). Control group rats injected with an equal volume of dextran showed normal level of iron (serum iron – 199.33 ± 19.96µg/dl). There was significant increase in serum ferritin level in iron overloaded group rats (4.04 ± 0.11 ng/dl) compared to normal control group rats (1.70 ± 0.08 ng/dl). All the studies were carried out for a period of 30 days. Blood, urine and fecal samples were collected on 15th and 30th days under fasting conditions **(Fig. 1).**

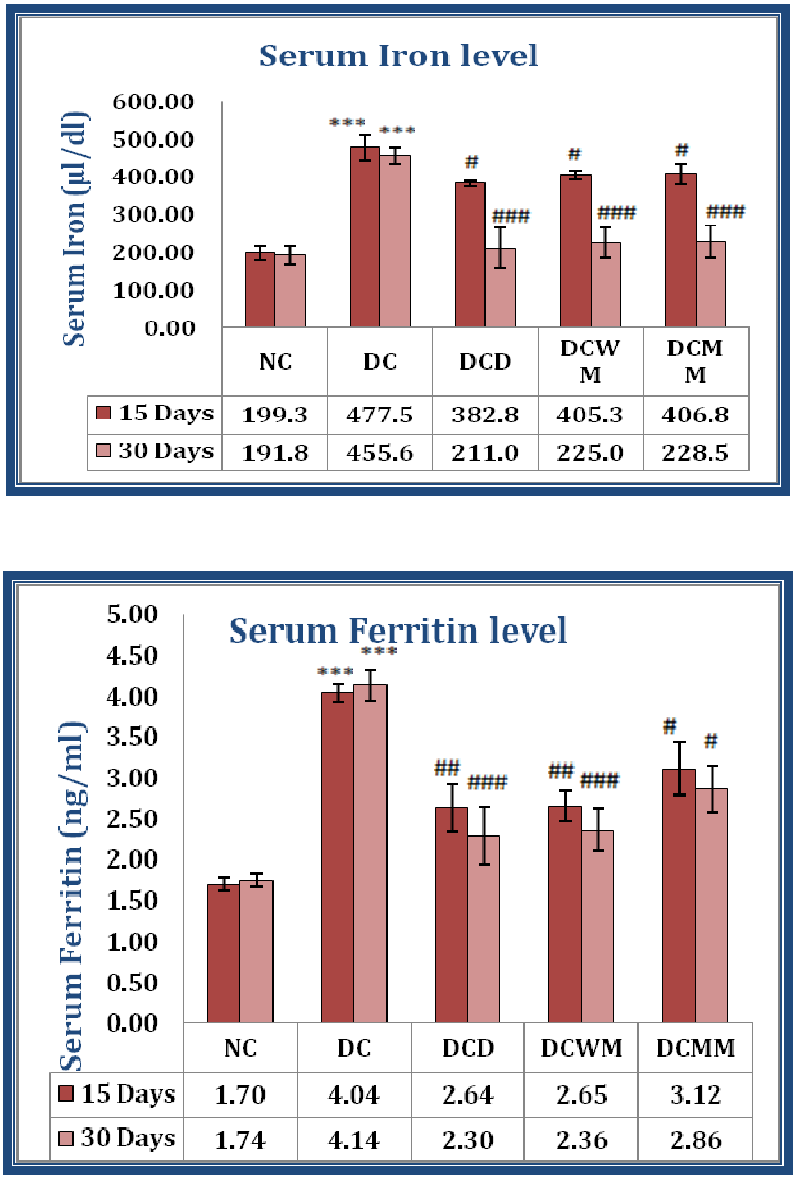


Fig.1: Beneficial Effects of Various Extracts of *M. sativa* on Iron over Load Rats with Respect to Changes in Parameters Related to its Mechanism of Action

Values are expressed as Mean ± S.E.M; *-significantly different from normal control (p< 0.001); # - significantly different from diseases control (p < 0.05); # #- significantly different from diseases control (p < 0.01);# ##- significantly different from diseases control (p < 0.001);NC: Normal control received dextrose solution;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day);DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day).**

After 15 days of treatment, there was significant reduction in serum iron and ferritin levels in desferoxamine group (serum iron – 382.83 ± 8.20µg/dl, serum ferritin – 2.64 ± 0.29 ng/dl). There were significant reduction in serum iron and ferritin levels after treatment with water extract group (serum iron - 405 ± 11.7 µg/dl, serum ferritin – 2.65 ± 0.18 ng/dl) and methanol extract group

(serum iron – 406.83 ± 25.72 µg/dl, serum ferritin – 3.12 ± 0.33 ng/dl) of *M. sativa* compared to disease group(Fig. 1).

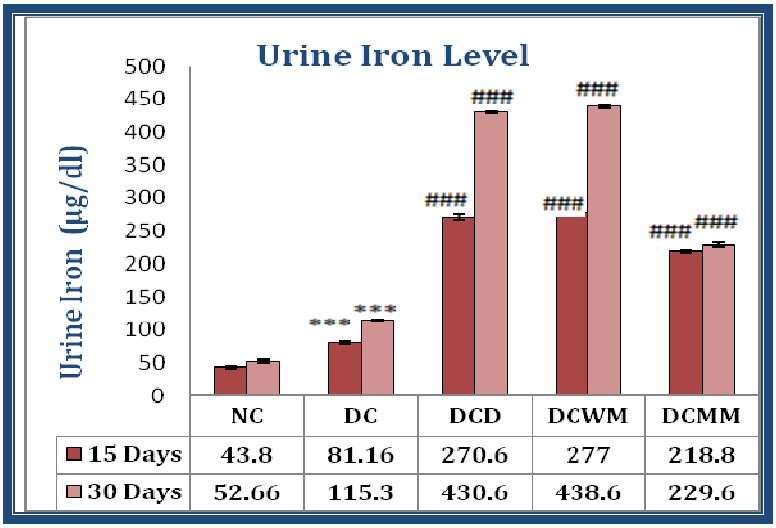
Thus, in- vivo findings in our study suggest that possibly the same iron chelator constituent is present in both methanol and water extracts (probably in higher concentration in water extract) and that this constituent has comparable iron chelating activity to that of desferoxamine **(Table 1).**

Table- 1: Beneficial Effects of Various Extracts of *M. sativa* on Iron Overload Rats with Respect to Changes in Parameters Related to its Mechanism of Action**.**

Parameters	NC (n=6)		DC (n=6)		DCD (n=6)		DCWM (n=6)		DCMM (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Serum Iron µg/dl	199.3 ± 19.9	191.8 ± 25.5	477.5 ± 34.6 ***	455.6 ± 22.8 ***	382.8 ± 8.20 #	211 ± 52.9 ###	405.3 ± 11.7 #	225 ± 39.6 ###	406.8 ± 25.72 #	228.5 ± 43.41 ###
Serum Ferritin mg/dl	1.70 ± 0.08	1.75 ± 0.05	4.04 ± 0.11 ***	4.14 ± 0.18 ***	2.64 ± 0.29 ##	2.30 ± 0.35 ###	2.65 ± 0.18 ##	2.36 ± 0.26 ###	3.12 ± 0.33 #	2.86 ± 0.28 #

Values are expressed as Mean ± S.E.M;*-significantly different from normal control (p< 0.001); # - significantly different from diseases control (p < 0.05);# #- significantly different from diseases control (p < 0.01); ### - significantly different from diseases control (p < 0.001);NC: Normal control received dextrose solution ;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day)**

Urine iron and faces iron levels: No changes were observed in urine and fecal iron in iron overloaded group rats (urine iron – 115.3 ± 1.9 µg/dl, faces iron – 3.9 ± 0.13 µg /dl) and placebo group (urine iron – 52.6 ± 2.1 µg/dl, faces iron – 2.5 ± 0.09 µg /dl), because iron does not excrete from body. There was significant increase in urine iron and faces iron levels in desferoxamine group (urine iron – 430.6 ± 2.2 µg/dl, faces iron – 9.5 ± 0.12 µg /dl), water extract group (urine iron – 438.6 ± 2.2 µg/dl, faces iron – 6.3 ± 0.19 µg /dl) and methanol extract group (urine iron – 229.6 ± 2.8 µg/dl, faces iron – 5.6 ± 0.14 µg /dl) compared to iron overloaded group rats (urine iron – 115.3 ± 1.9 µg/dl, faces iron – 3.9 ± 0.13 µg /dl) **(Fig. 2)**



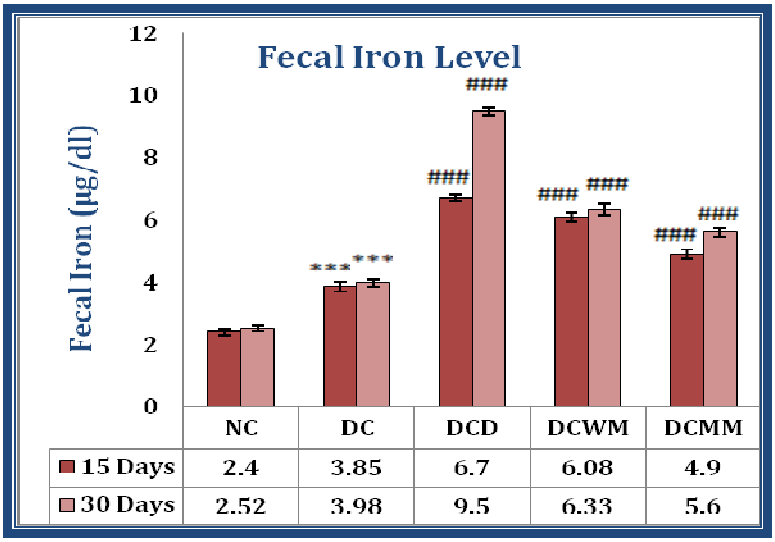


Fig.2: Beneficial Effects of Various Extracts of *M. sativa* on Iron Overload Rats with Respect to Excretion of Iron in Urine and Feces.

Values are expressed as Mean ± S.E.M; *-significantly different from normal control (p< 0.001);# ##- significantly different from diseases control (p < 0.001);NC: Normal control received dextrose solution;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day).**

Increase in urine and fecal excretion of iron in rats treated with water and methanol extracts of alfalfa indicate iron chelating property of *M. sativa* that was comparable to desferoxamine group. The beneficial effects observed after 15 and 30 days treatment period with *M. sativa* in iron overloaded rats (Table 2). These data suggest effectiveness of water and methanol extracts in reduction of iron overload in rats by increase iron excretion in urine and feces.

These data suggest water and methanol extracts have effectiveness in reduction of iron overload which may be benefits in iron overload disorders as desferoxamine **(Table 2).**

Table- 2: Beneficial Effects of Various Extracts of *M. Sativa* on Iron Overload Rats with Respect to Excretion of Iron in Urine and Feces.

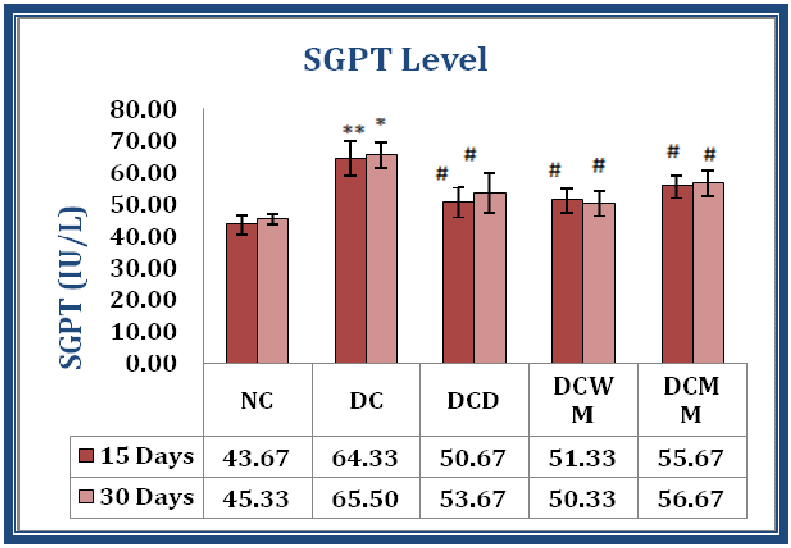
Parameter	NC (n=6)		DC (n=6)		DCD (n=6)		DCWM (n=6)		DCMM (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Urine Iron (µg/dl)	43.8 ± 1.7	52.6 ± 2.1	81.1 ± 2.4 ***	115.3 ± 1.9 ***	270.6 ± 3.8 ###	430.6 ± 2.2 ###	277 ± 2.04 ###	438.6 ± 2.2 ###	218.8 ± 2.1 ###	229.6 ± 2.8 ###
Faces Iron (µg/dl)	2.4 ± 0.11	2.5 ± 0.09	3.85 ± 0.16 ***	3.98 ± 0.13 ***	6.7 ± 0.11 ###	9.5 ± 0.12 ###	6.08 ± 0.14 ###	6.3 ± 0.19 ###	4.9 ± 0.14 ###	5.6 ± 0.14 ###

Values are expressed as Mean ± S.E.M; *-significantly different from normal control (p< 0.001);# ##- significantly different from diseases control (p < 0.001);NC: Normal control received dextrose solution ;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.);DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day).**

Protective effects of *M. sativa* on iron overload complications on vital organs: Excess iron in vital organs, even in mild cases of iron overload, increases the risk for liver disease (cirrhosis, cancer), kidney diseases, heart attack or heart failure, diabetes mellitus etc. and in some cases premature death.

SGPT and SGOT levels: There were significant increases in SGPT (64.3 ± 5.27 IU/L) and SGOT (167.8 ± 5.1 IU/L) levels in iron overloaded group as compared to normal control group (SGPT – 43.6 ± 3.1 IU/L, SGOT -131.0 ± 4.12 IU/L). After treatment with water and methanol extracts of *M. sativa* there was significant reduction in these enzyme levels (water extract SGPT – 51.3 ± 3.83 IU/L, SGOT – 133.83 ± 4.9 IU/L; methanol extract SGPT – 55.6 ± 3.6 IU/L, SGOT – 150.5 ± 2.2 IU/L) indicating protective effects of extracts in liver complications due to iron overload (Table 3)**(Fig. 3).** Thus, we can say that, *Medicago sativa* improves liver and cardiac function in iron overload.

Serum Creatinine and Creatinine Kinase Levels: Serum creatinine and creatinine kinase levels were significant increased in iron overloaded rats group (serum creatinine- 1.03 ± 0.08 mg/dl and creatinine kinase- 998.5 ± 128.0 µg/l) as compared to placebo group (serum creatinine- 0.73 ± 0.04 mg/dl and creatinine kinase- 448.1 ± 9.55 µg/l). Methanol and water extracts treated animals showed reduction in levels of these enzymes (water extract, serum creatinine- 0.78± 0.09 mg/dl and creatinine kinase- 559.0 ± 51.08 µg/l; methanol extract, serum creatinine- 0.83 ± 0.04 mg/dl and creatinine kinase- 576.8 ± 22.38 µg/l) indicating that these extracts prevent damage to vital organs like kidney and heart in iron overload complications **(Table 3) (Fig. 4).** Thus, we can say that, *Medicago sativa* improves kidney and cardiac function in iron overload condition.



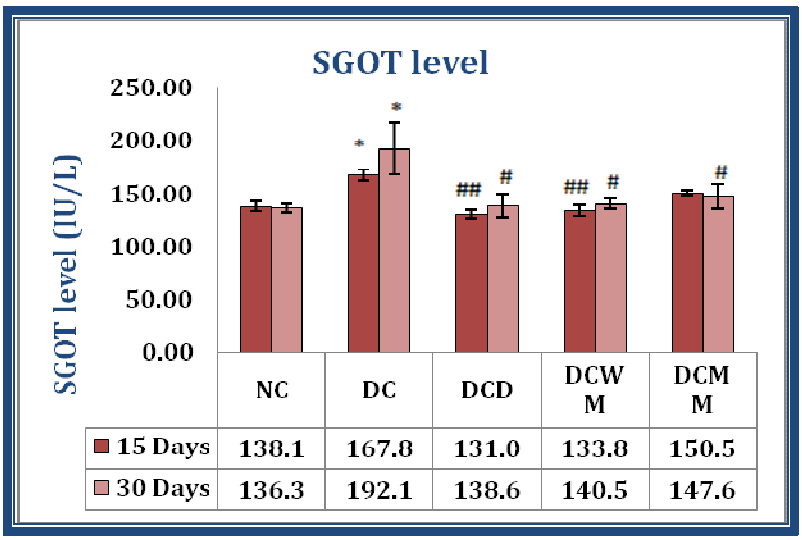


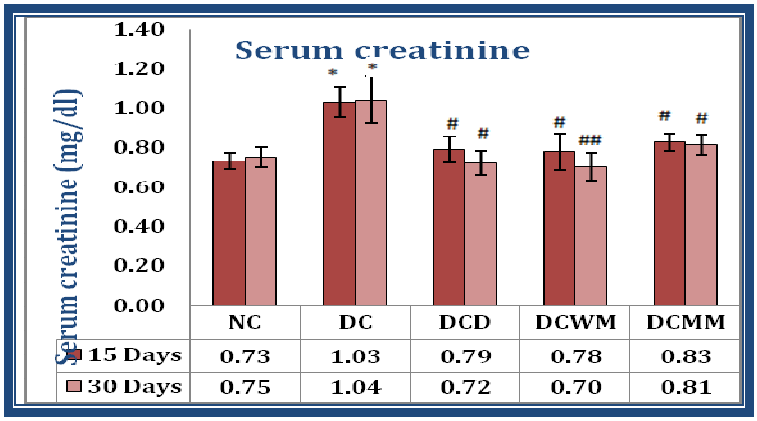
Fig. 3: Beneficial Effect of Various Extracts of *M. sativa* on Iron Overload Induce Liver and heart Complication.

Values are expressed as Mean ± S.E.M; *- significantly different from normal control (p < 0.05); **-significantly different from normal control (p< 0.01);# - significantly different from diseases control (p < 0.05);# #- significantly different from diseases control (p < 0.01);NC: Normal control received dextrose solution ;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day) .

Table- 3: Beneficial Effect of Various Extracts of *M. sativa* on Iron Overload Complications on Major Organ Heart, Liver and Kidney.

Parameter	NC (n=6)		DC (n=6)		DCD (n=6)		DCWM (n=6)		DCMM (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
SGPT IU/L	43.6 ± 3.16	45.3 ± 1.65	64.3 ± 5.23 **	65.5 ± 4.04*	50.6 ± 4.87#	53.6 ± 6.24 #	51.3 ± 3.83#	50.3 ± 3.90 #	55.6 ± 3.66#	56.6 ± 3.87#
SGOT IU/L	138.1 ± 4.6	134.8 ± 4.6	167.8 ± 5.1*	189.3 ± 24.8*	131.0 ± 4.12 ##	138.6 ± 10.6 #	133.83 ± 4.9##	150.8 ± 3.7#	150.5 ± 2.2 #	147.6 ± 11.6#
Serum creatinine mg/dl	0.73 ± 0.04	0.75 ± 0.05	1.03 ± 0.08*	1.04 ± 0.12*	0.79 ± 0.07#	0.72 ± .06#	0.78 ± .09#	0.70 ± .07# #	0.83 ± 0.04 #	0.81 ± 0.05#
Creatine kinase (CKMB) µg/l	448.1 ± 9.55	538.6 ± 41.24	998.5 ± 128.0 ***	1047.3 ± 63.27 ***	549.8 ± 29.76 ###	536.2 ± 25.72## #	559.0 ± 51.08 ###	509.3 ± 38.3 ###	576.3 ± 22.38 ###	581.8 ± 21.02 ###

Values are expressed as Mean ± S.E.M;*- significantly different from normal control ($p < 0.05$);-significantly different from normal control ($p< 0.01$);***-significantly different from normal control ($p< 0.01$);# - significantly different from diseases control ($p < 0.05$);# #-significantly different from diseases control ($p < 0.01$);# ##- significantly different from diseases control ($p < 0.001$);NC: Normal control received dextrose solution;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day);DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day).**



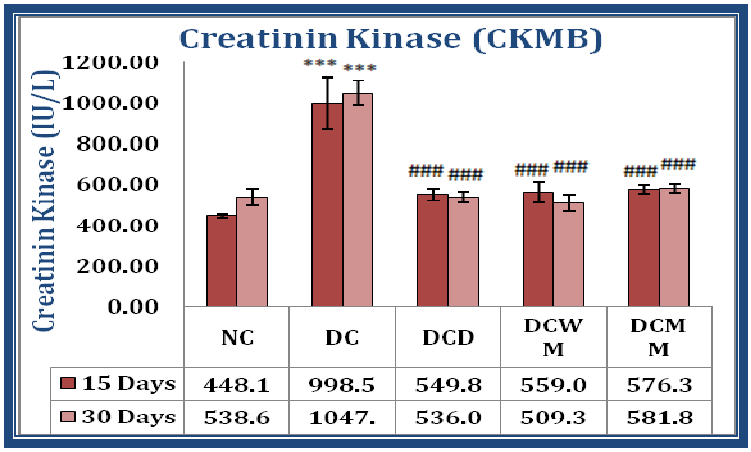


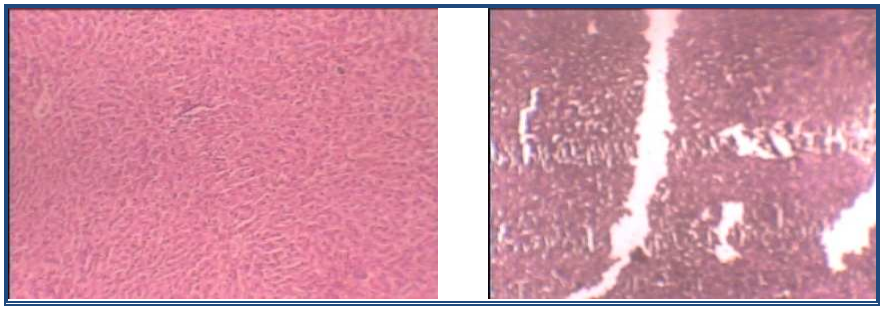
Fig. 4: Beneficial Effects of Various Extracts of *M. sativa* on Iron Overload Induce Kidney and Cardiac Complications.

Values are expressed as Mean ± S.E.M;*- significantly different from normal control (p < 0.05);*-significantly different from normal control (p< 0.01);# - significantly different from diseases control (p < 0.05);# #- significantly different from diseases control (p < 0.01);# ##- significantly different from diseases control (p < 0.001);NC: Normal control received dextrose solution;DC: Disease control treated with iron dextran (12.5mg/l00g body wt.) ;DCD: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) ;DCWM: Disease control treated with water extract of *M. sativa* (500 mg/kg, p.o., per day)**

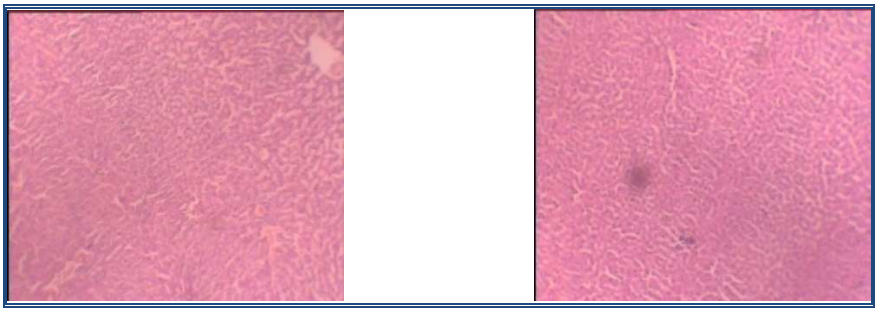
DCMM: Disease control treated with methanol extract of *M. sativa* (250 mg/kg, p.o., per day)

Histopathological Study of Liver, Kidney and Heart: Hepatotoxicity is the most common finding in iron overload because liver is the main recipient of the excess iron[28].

Results of histopathological study of liver suggested that chronic treatment with desferoxamine and water and methanol extracts of *M. sativa* reduce iron pigmentation, pleomorphism, vaculation, fibrosis, disarrangement and degeneration of hepatocytes as compared to iron overloaded group animals **(Fig. 5).**

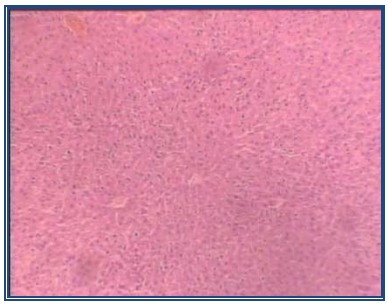


(a)Normal control **(b).Iron overloaded Disease control**



(c). Iron overloaded rats Treated with Desferoxamine

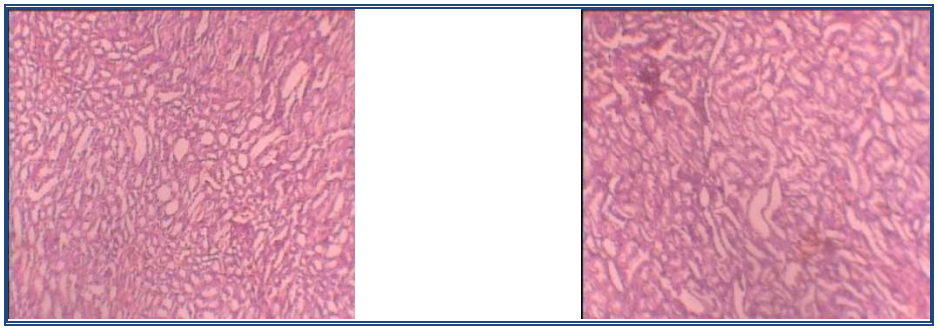
(d.) Iron overloaded rats treated with Water extract



(e.) Iron overloaded rats treated with Methanol extract

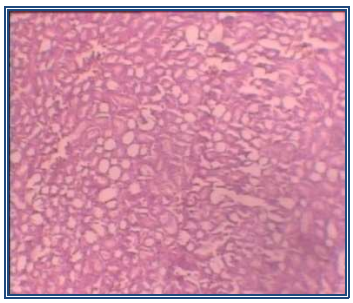
Fig. 5: Protective Effects of Various Extracts of *M. sativa* on Iron Overloaded Liver Complications**.**

Normal structure of the cortex and medulla was observed in the kidney of normal control rats (Fig. 6). The animals exposed to chronic iron dextran showed damage of renal tubules and glomeruli. Hypertrophy of epithelial cells and degeneration of epithelia of renal tubules with infiltration of mononuclear cells, dilation of glomerul and mononuclear cell infiltrates were evident in all diseases control rats (Fig. 6). Pathological changes in kidney ultra structure (injured brush-border microvilli and swollen proximal convoluted tubular cells) were observed when iron dextran. Histology of kidney in iron overload group rat treated with desferoxamine, an iron chelator, showed reduced damage of renal tubules and glomeruli. Pathological changes were also prevented by desferoxamine. Our result suggest protective effects of methanol and water extracts of *M. sativa* in iron overload kidney complications as it reduced damage of kidney ultra structure (injured brush-border microvilli and swollen proximal convoluted tubular cells). Protective effects found much better in methanol extract and less in water extract **(Fig. 6).**



(a)Normal control **(b).Iron overloaded Disease control**

(c). Iron overloaded rats treated with Desferoxamine **(d.) Iron overloaded rats treated with Water extract**



(e.) Iron overloaded rats treated with Methanol extract

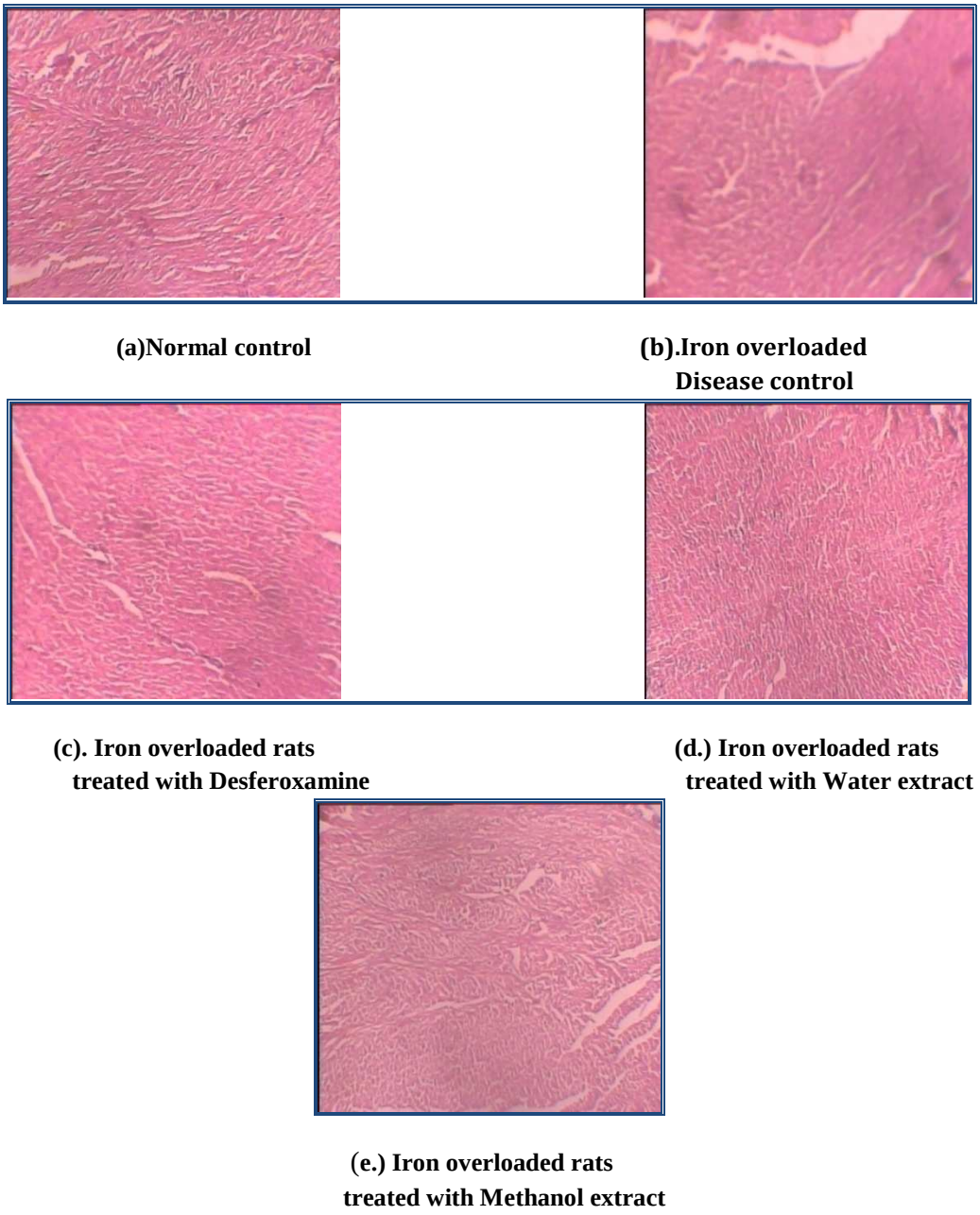
Fig. 6: Protective Effects of Various Extracts of *M. sativa* on Iron Overloaded Kidney Complication**s.**

Iron-overload cardiomyopathy is a common cause of CV death worldwide in subjects in their second and third decades of life [29]. Indeed, iron-overload cardiomyopathy is the most important determinant of survival in European[30], North American[31], and Chinese patients with thalassemia major. Long-term follow-up studies in beta-thalassemia patients have established that the level of cardiac iron accumulation correlates directly with both the occurrence of heart disease and mortality, while in

patients with primary haemochromatosis, CV disease also contributes significantly to their mortality and morbidity[32].

Hearts from rat injected chronically with iron displayed extensive interstitial fibrosis and myocyte vacuolar degeneration with mild inflammatory infiltrate compared to placebo (Fig.7) there was vascular hemorrhage and hypertrophy observed in iron overload rats compared to placebo.

Treatment with desferoxamine and extracts of methanol and water of *M. sativa* showed protective effects on myocytes as well as reduces fibrosis and hypertrophy of myocytes. Vascular hemorrhages were also found to be reducing in iron overloaded group rats treated with methanol and water extracts **(Fig. 7).**



(a)Normal control

(b).Iron overloaded Disease control

(c). Iron overloaded rats treated with Desferoxamine

(d.) Iron overloaded rats treated with Water extract

(e.) Iron overloaded rats treated with Methanol extract

Fig. 7: Protective Effects of Various Extracts of *M. sativa* on Iron Overloaded Cardiac Complications

CONCLUSION

We have confirmed *in-vivo* iron chelating activity of *Medicago sativa* (Alfalfa) and its various extracts. The data suggest that *Medicago sativa* have chelating power nearer to synthetic iron chelating agent desferoxamine. The mechanism of iron chelating activity of *Medicago sativa* seems to be the increase in excretion of iron through urine as well as faeces and that the chelate complexation process with iron is reversible. Our data suggest that *Medicago sativa* protects vital organs like liver, kidney and heart in iron over load conditions like thalassemia and other iron overload disorders.

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