



ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2010, 2(6):275-283

***In vivo* antioxidant activity of *Moringa oleifera* leaf and pod extracts against carbon tetra chloride induced liver damage in albino mice**

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ABSTRACT

Study was conducted to test the hypothesis that Moringa oleifera Lam. Leave and pod has antioxidant activity. For this purpose study was designed using different concentration of crude aqueous and ethanol (alcoholic) extracts of Moringa oleifera Lam. Leaves and pods respectively to investigate protection against CCl₄ induced hepatocytes injury of mice in vitro and compared with standard silymarin . Results shows that extract was effective in the reducing CCl₄ induced enhanced activities of SOD, GLU, Catalase, lipid peroxidation and % viability. Data shows that CCl₄ treatment decreased SOD,catalase,glutathioe,and peroxidase while increased lipids oxidation and MDA is the by product of lipid peroxidation so increases.Pretreatment with 500 mg/kg, 750 mg/kg,1000 mg/kg(p.o.) of Moringa oleifera hydro- alcoholic leaf extract and 500 mg/kg, 750 mg/kg,1000 mg/kg(p.o.) of Moringa oleifera aqueous pods extract improved the SOD, catalase,glutathione,and peroxidase levels significantly(p>0.05) and reduced lipids peroxidationThis shows hepatocellular damage caused by CCl₄ and its recovery by pretreatment with the crude extract of leaves and pods suggest that it might be considered as a potential source of natural antioxidant agent, which could be related to the free radical scavenging properties of various components present in varying concentration in the extract which is evident from the free radical measurement.

Key Words: Carbon tetra chloride, Moringa oleifera, Antioxidant activity, Silymarin.

INTRODUCTION

The plant *Moringa oleifera* Lam (Moringaceae) is the most widely cultivated variety of the genus *Moringa* and is distributed in the sub-Himalayan ranges of India, Sri-Lanka, Mexico, Arabia and

South Western Africa. The leaves and pods of *Moringa oleifera* remove all kinds of pain, good vesicent, expectorant, stimulant and abortifacient. The decoction of the leaf is used as a stimulant, analgesic and diuretic. The pods are edible, seeds are useful as purgative, antipyretic, cures eye diseases, head complaints and are used in venereal affections. Leaf and pod of *Moringa oleifera* contains Flavonoid, phenolic acid, and phenolic diterpenes, lignane are the example of phenolic compounds with antioxidants properties. The present study was undertaken to screen the antioxidant activity of the leaf and pod of *Moringa oleifera*. [1] [2]

EXPERIMENTAL SECTION

The leaf and pod of *Moringa oleifera* were collected from the local areas of Udaipur district, Rajasthan, India during February 2010 and were authenticated by Dr. S. S. Katewa, Department of Botany, M. B. College, Udaipur Rajasthan.

Preparation of Extracts

Leaves and fruits (pods) of *M. oleifera* will be ground separately in a mortar. Each of the plant tissues will be soaked in approximately 400ml of 95% ethanol and water on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whatman filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50°C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4°C. The prepared extract was weighed and mixed with known concentration of ethanol. The extract will be subjected to photochemical screening and administered to the animals in the course of this study. [3]

Animals

Healthy albino mice of either sex and of approximately the same age, weighing about 20-30 gm were used for the study. They were fed with standard chow diet and *ad libitum*. They were housed in polypropylene cages maintained under standard condition (12 hour light, 12 hour dark cycle; 25 ± 3°C, 35-60 % humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting. The acute toxicity studies of ethanol and aqueous extracts were carried out according to OECD guidelines. 1000 mg/kg dose of both the extracts was found non-toxic in mice and was taken for the further study.

Antioxidant Activity

Male albino mice of Swiss strain weighing between 20-30 g will be used and maintained on normal diet and water at *libitum*, The animals were divided into nine groups of six animals each.[5]

Group-1 animal served as control, treated with distilled water. **Group-2** animal served as hepatotoxic control, treated with CCL4 in a dose of 1 ml/kg, ip., on alternate day for seven days.

Group-3 animals served as a standard group, and were administered Silymarin in a dose of 100 mg/kg, po daily and CCL4 in a dose of 1 ml/kg, ip., on alternate day for seven days.

Group-4 animal were treated with daily dose of 1000 mg/kg, po., *Moringa oleifera* Leaf extract daily and CCL4 in a dose of 1 ml/kg, ip., on alternate day for seven days.

Group-5 animal were treated with daily dose of 750 mg/kg, po., *Moringa oleifera* Leaf extract daily and CCL4 in a dose of 1 ml/kg, ip., on alternate day for seven days

Group-6 animal were treated with daily dose of 500 mg/kg, po., *Moringa oleifera* Leaf extract daily and CCL4 in a dose of 1 ml/kg, ip., on alternate day for seven days.

Group-7 animal were treated with daily dose of 1000 mg/kg ,po., *Moringa oleifera* pods(fruit) extract daily and CCL4 in a dose of 1 ml/kg,ip., on alternate day for seven days.

Group-8 animal were treated with daily dose of 750 mg/kg ,po., *Moringa oleifera* pods(fruit) extract daily and CCL4 in a dose of 1 ml/kg,ip., on alternate day for seven days.

Group-9 animal were treated with daily dose of 500 mg/kg ,po., *Moringa oleifera* pods(fruit) extract daily and CCL4 in a dose of 1 ml/kg,ip., on alternate day for seven days.

On eighth day animals were sacrificed by using ether anaesthesia and liver were rapidly excised and rienceed in ice-cold salien.

Preparation of homogenate

Tissue rienceed with 0.9% ice-cold normal salien and 10% w/v homogenate was prepared using 0.1 N Tris HCL buffer (pH 7.4),centrifused at 10000 rpm, for 20 min at 4 C.The supernatent was obtained.

This supernatent was used for the estimation of melonialdehyde(MDA) catalase(CAT) superoxide dismutase(SOD) glutathione(GLU). [6] [7] [8] [9]

Statistical analysis

Results expressed as mean \pm S.E., were evaluated by unpaired student T test. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The present study is based on the comparision of the prevention of oxidative stress produced by carbontetrachloride by silimarin and different doses of *Moringa Olieifera* extract.Data shows that CCl₄ treatment decreased SOD,catalase,glutathioe,and peroxidase while increased lipids oxidation and MDA is the by product of lipid peroxidation so increases.Pretreatment with 500 mg/kg, 750 mg/kg,1000 mg/kg(p.o.) of *Moringa oleifera* hydro- alcoholic leaf extract and 500 mg/kg, 750 mg/kg,1000 mg/kg(p.o.) of *Moringa oleifera* aqueous pods extract improved the SOD, catalase,glutathione,and peroxidase levels significantly($p > 0.05$) and reduced lipids peroxidation. SOD is aubiquitous enzyme that dismutates superoxide radical to H₂O₂ and oxigen so is one of the chief defence mechanism. The H₂O₂ formed by SOD and other processes is scavenged by catalase that catalyzes the dismutation of H₂O₂ into water and moleculer oxygen. Thus,the antioxidant enzyme catalase is responceible for detoxfication of H₂O₂. Glutathione is a tripeptide of glycine,glutamic acid,and cysteine. Glutathione is an important naturally occuring antioxidant as it prevents hydrogen of sulphhydl group to be abstracted insteads of methylene hydrogen of unsaturated lipids. Therefor level of glutathione are of critical importance in tissue injury couosed by toxic substances. The antioxidant enzyme and glutathione forms the first line of defence againced free radical-induced damage,offer protection against free radical, and thereby maintained the low level of lipild peroxide. Peroxidase is an enzyme that catlyze the reduction of hydroperoxide, incuding hydrogen peroxide,and function yo protect the cell from peroxidative damage.

As the *Moringa oleifera* hydro- alcoholic leaf extract in dose of 1000 mg/kg and *Moringa oleifera* aqueous pods extract in dose of 750 mg/kg improved the SOD, catalase,glutathione,and peroxidase levels significantly ($p > 0.05$) and redused lipids peroxidation, which were comparable with Silymarine 100 mg/kg The present study shows elevated level of antioxidant in drug treated grous, whereas decreased in induced and controled groups. The protien level was

also increased in drug treated groups in comparison with induced group of experimental analysis. The lipids peroxidation was highly elevated in induced groups then the controlled and drug treated groups.

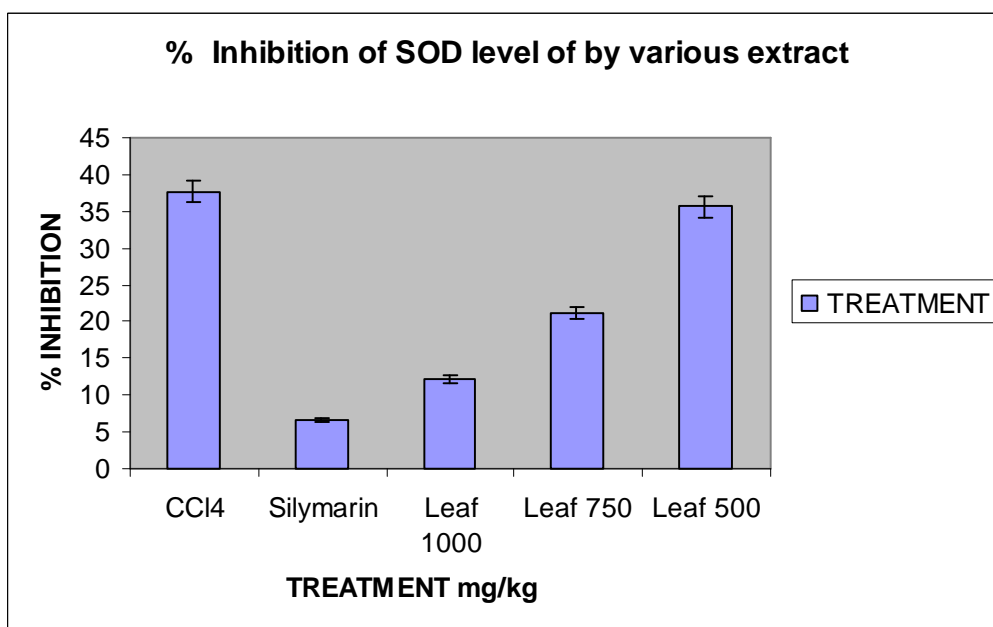
The conclusion of the present study shows that the *Moringa oleifera* hydro- alcoholic leaf extracts (1000 mg/kg) and *Moringa oleifera* aqueous pod (fruit) extract (750 mg/kg) contain high amount of tannin, phenolic compounds, and flavonoids. The poly phenolic constituents of this plants could be contributory to their ethano medical use. Thus it can be concluded that extracts of *Moringa oleifera* produce significant antioxidant activity. Alcoholic extracts of Leave have much lesser antioxidant activity than the Aqueous extract. However direct studies using more fractioned extract could provide better evidence for this claim.

TableNo.1: Inhibition of various parameters compared to control= 100% of leaves extract of *Moringa oleifera*

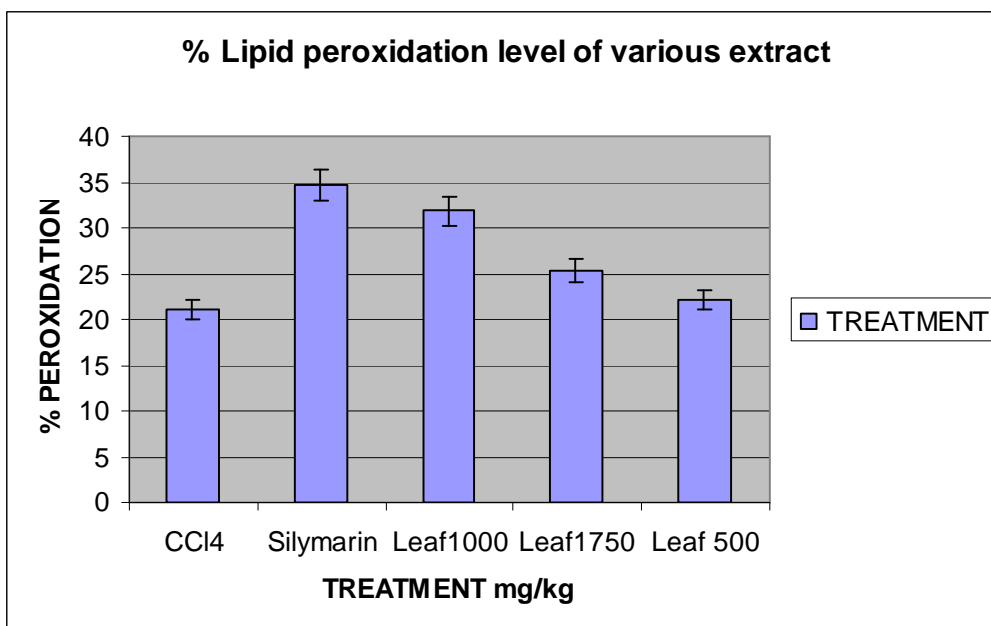
Treatment Groups	Dose (mg/kg)	glutathione % Inhibition of enzyme	Melonaldehyde % Lipid peroxidation	SOD % inhibition of enzyme	Catalase % inhibition of enzyme
CCl ₄	1(ml/kg)	36.21±0.26*	21.06±0.09*	37.68±0.59*	26.92±0.86*
Silymarin	100	06.70±0.39*	34.66±0.11*	06.50±0.69*	04.30±0.57*
Leaves	1000	17.40±0.75*	31.90±0.08*	12.10±0.69*	10.56±0.11*
Leaves	750	24.46±0.25*	25.23±0.07*	21.23±0.65*	14.72±0.42*
Leaves	500	31.44±0.52*	22.17±0.05	35.61±0.47*	17.22±0.12*

Results expressed as Mean±SEM n=6 *p<0.05, Significant difference compared with control
% Inhibition of various parameters compared to control= 100% of leaves extract of *Moringa oleifera*:

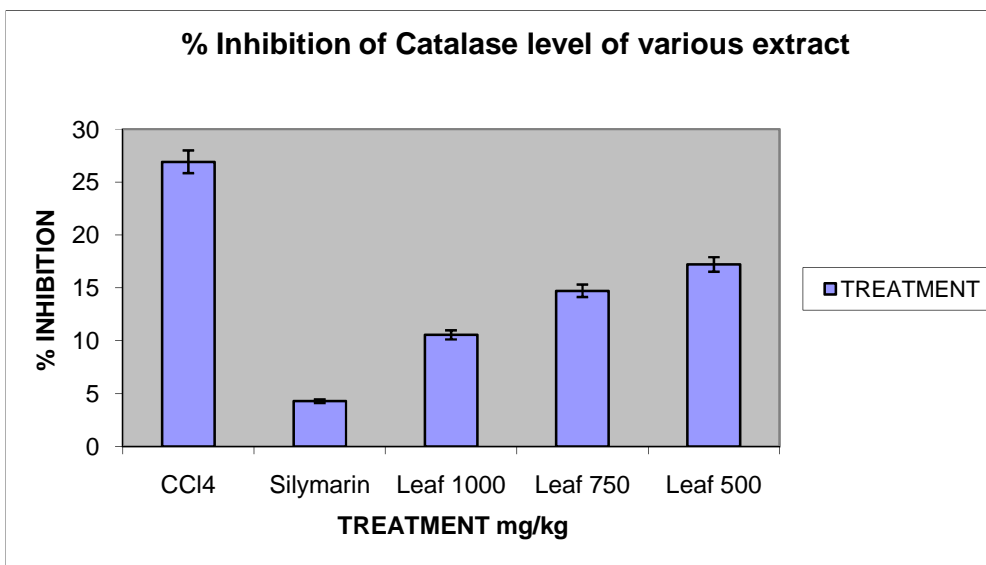
Graph No.1:-% Inhibition of SOD by leaves extract of *Moringa oleifera*



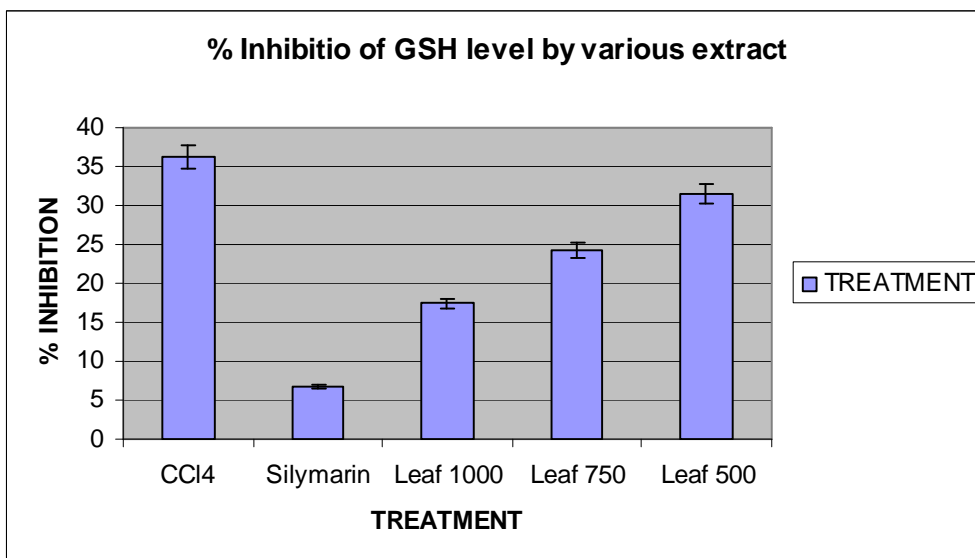
Graph No.2:-% Inhibition of MDA by leaves extract of *Moringa oleifera*



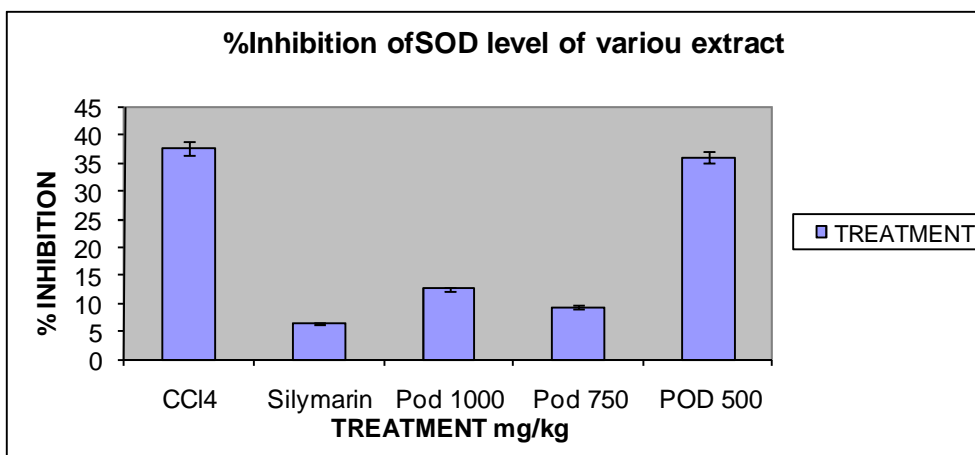
Graph No. 3:-%Inhibition of CAT by leaves extract of *Moringa oleifera*



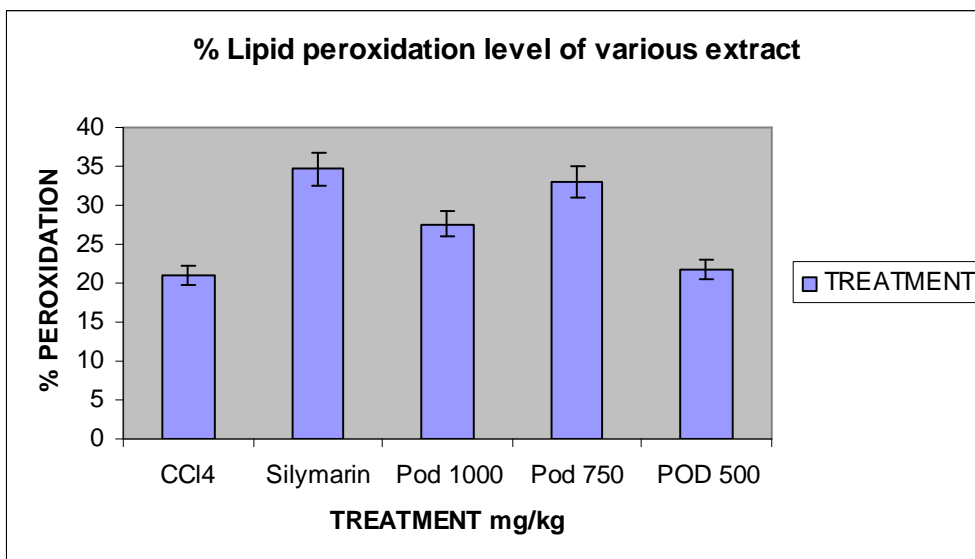
Graph No.4:-%Inhibition of GSH by leaves extract of *Moringa oleifera*



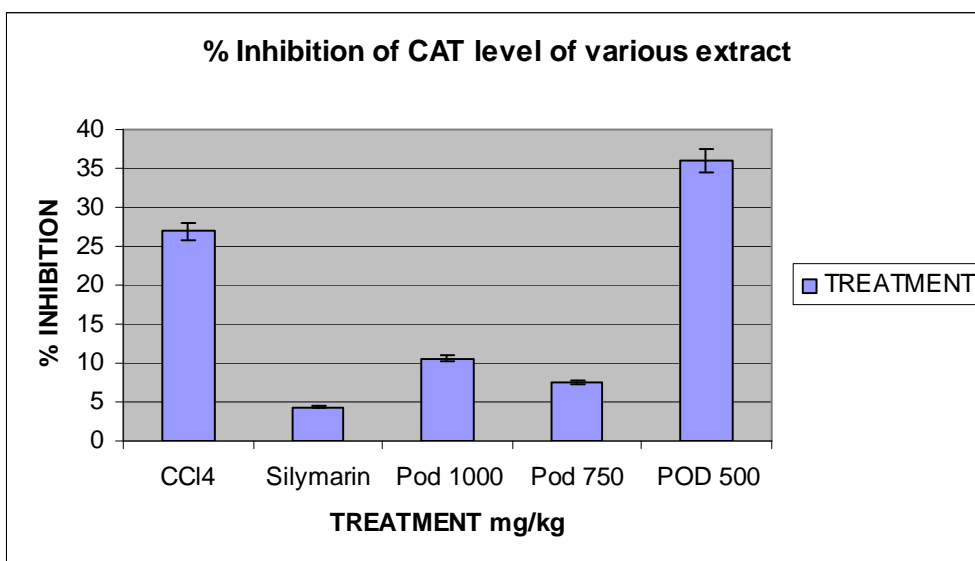
Graph No.5:-% Inhibition of SOD by PODS extract of *Moringa oleifera*

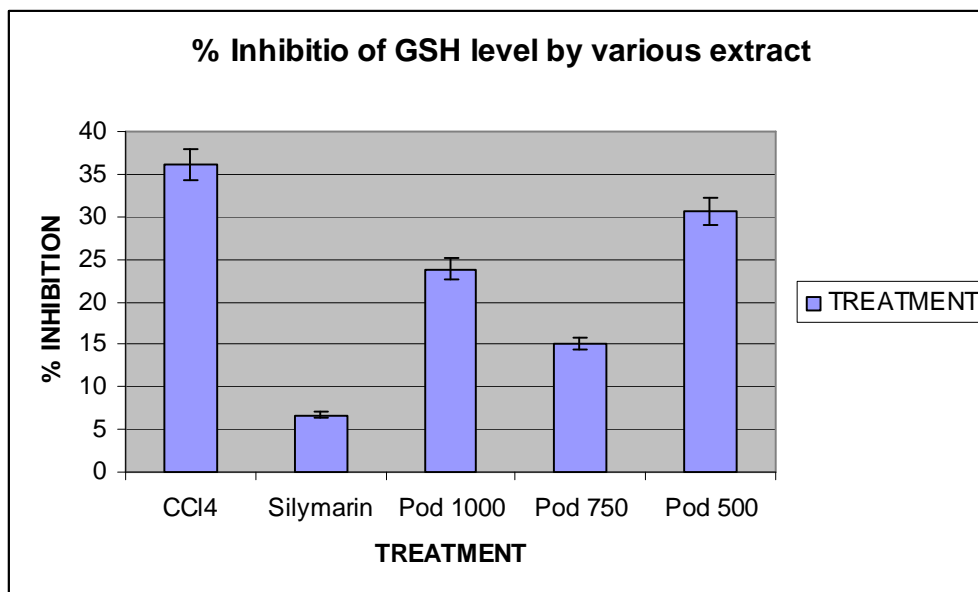


Graph No.6:-% Inhibition of MDA by PODS extract



Graph No.7:-%Inhibition of CAT by PODS extract of Moringa oleifera



Graph No 8:-% Inhibition of GSH by PODS extract of *Moringa oleifera*TableNo.2: % Inhibition of various parameters compared to control= 100% of pods extract of *Moringa oleifera*

Treatment Groups	Dose (mg/kg)	glutathione %Inhibition of enzyme	Melonaldehyde % Lipid peroxidation	SOD %inhibition of enzyme	Catalase %inhibition of enzyme
CCl ₄	1(ml/kg)	36.21±0.26*	21.06±0.09*	37.68±0.59*	26.92±0.86*
Silymarin	100	06.70±0.39*	34.66±0.11*	06.50±0.69*	04.30±0.57*
Pods	1000	23.86±0.42*	27.59±0.13*	12.65±0.99*	10.56±0.11*
Pods	750	15.11±0.32*	33.11±0.04*	09.40±0.35*	07.54±0.35*
Pods	500	30.59±0.12*	21.77±0.12*	36.02±0.37*	17.22±0.12*

Results expressed as Mean± SEM n=6 *p<0.05 Significant difference compared with control
% Inhibition of various parameters compared to control= 100% of PODS extract of *Moringa oleifera*

Acknowledgements

This research was supported by the B.N. College of Pharmacy, Udaipur. The author wish to thank Dr. S. S. Sisodia, HOD, Department of Pharmacology, B. N. College of Pharmacy, Udaipur, for his valuable suggestion during my work.

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