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# Hepatoprotective Effect of *Macrotyloma uniflorum* Seeds (Horse gram) in Carbon Tetrachloride-Induced Liver Toxicity in Rats: Evidence of an Underlying Antioxidant Activity

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**Abstract:** The protective effects of a hydroalcoholic extract of seeds of *Macrotyloma uniflorum* (MUSE) on carbon tetrachloride ($CCl_4$) induced hepatotoxicity and the probable mechanism(s) involved in this protection were investigated in rats. Liver damage was induced in Wistar rats by administering 1:1 (v/v) mixture of $CCl_4$ and olive oil (1 ml/kg, i.p.) once daily for 7 days. MUSE (250 mg/kg & 500 mg/kg) and the reference drug silymarin (100 mg/kg) were administered orally for 10 days to $CCl_4$ treated rats, this treatment beginning 3 days prior to the commencement of $CCl_4$ administration. The degree of protection was evaluated by determining the marker enzyme (AST, ALT & ALP) activities and levels of bilirubin, albumin and total proteins in serum. Further, the effect of MUSE on malondialdehyde (lipid peroxidation marker) content, glutathione levels and superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities was estimated in liver homogenate to evaluate its antioxidant activity. MUSE attenuated significantly the $CCl_4$-elevated levels of AST, ALT, ALP, malondialdehyde and bilirubin. MUSE also restored significantly the $CCl_4$-depleted levels of albumin, total protein, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. MUSE 500 mg\kg demonstrated greater hepatoprotection than MUSE 250 mg\kg. The present findings indicate that the hepatoprotective effect of MUSE in $CCl_4$ induced oxidative damage may be due to an augmentation of the endogenous antioxidants and an inhibition of lipid peroxidation in liver.

**Key words:** *Macrotyloma uniflorum* seed, carbon tetrachloride, hepatoprotective, antioxidant activity.

## Introduction

Liver injury induced by chemicals has been recognized as a toxicological problem for a long period of time. Chemically induced liver injury occurs mostly because of liver's anatomical proximity to blood supply from the digestive tract, its ability to accumulate and metabolize foreign chemicals and its role in the excretion of xenobiotics or their metabolites into the bile.

Carbon tetrachloride ($CCl_4$), a well established hepatotoxin is biotransformed by hepatic microsomal cytochrome P450 (CYP) 2E1 to its active metabolite, the trichloromethyl ($CCl_3^{\cdot}$) radical, which readily reacts with oxygen to form a trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$)[1]. Antioxidants in the body such as reduced

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glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) play a pivotal role in neutralizing these harmful metabolites [2]. However, overproduction of the trichloromethyl free radical is considered as the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell apoptosis and necrosis [3,4,5].

*Macrotyloma uniflorum* (Family: Fabaceae) is a lesser known drought resistant legume grown throughout Asia, Africa and Australia and primarily utilized as feed to animals and horses. In India it is known as the "poor man's pulse" and used as a staple food. The *Macrotyloma* seeds also known as horse gram are small, ovoid and greyish brown or light reddish brown in colour with faint mottles and small scattered black spots on them [6]. Traditionally, horse gram has been used to treat various ailments such as haemorrhoids, tumors, bronchitis, splenomegaly, heart disease, leukoderma, urinary discharge, obesity, diabetes and asthma [7]. Recently, it has also been investigated for antimicrobial and haemolytic activities [8]. The soup prepared from horse gram is considered to be a useful remedy for treating common cold, throat infections, fever and urolithiasis [9]. The seeds have been reported to have potent antioxidant activity [10].

Seeds of *M. uniflorum* contain varying amounts of carbohydrates, proteins, amino acids, lipids, phenolic compounds (3,4-dihydroxy benzoic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid, syringic acid and sinapic acid), flavonoids and tannins (quercetin, kaempferol and myricetin), fatty acids (hexanoic acid and hexadecanoic acid), phytosterols (stigmasterol and β-sitosterol), anthocyanidins (cyanidin, petudin, delphinidin and malvidin), saponins and minerals like iron, calcium and molybdenum [11,12]. Phenolic acids obtained from *M. uniflorum* are considered to be potent antioxidants which act by scavenging free radicals and reactive oxygen species [10].

With this background, the present study was carried out to investigate the hepatoprotective effect and a possible underlying antioxidant activity of the hydroalcoholic extract of the seeds of *M. uniflorum* by assaying various marker enzymes, antioxidant enzymes, lipid peroxidation and GSH in rats with $CCl_4$-induced liver injury.

## Materials and methods

### *Plant material*

The seeds of *M. uniflorum* were collected from the Pune region of Maharashtra, India and were authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai after matching with the existing specimen (accession no. AD-06 ).

The seeds were dried, powdered mechanically and defatted using petroleum ether. The dry defatted powder was then extracted in a Soxhlet apparatus with 70 % methanol as the solvent at 60°C. The dry extract was stored in an air-tight container for experimental use.

### Drugs and chemicals

Silymarin, epinephrine, 5, 5'-dithiobis (2-nitrobenzoic acid) - (DTNB) and trichloro acetic acid (TCA) were purchased from Sigma Chemical Co., St Louis, MO, USA. Thiobarbituric acid (TBA), reduced glutathione, oxidized glutathione and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were obtained from local sources and were of analytical grade.

### Experimental animals

Wistar albino rats (150-200 g) of either sex were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25±2°C) and light (12 h light/ 12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pune, India) and water *ad libitum*. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No.25/1999/CPCSEA) and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research.

### Preparation of test and reference drug solutions

The *Macrotyloma uniflorum* seed extract (MUSE) was dissolved in distilled water and the aqueous extract solution was used for adminis-

tration. Silymarin was suspended in an aqueous solution of 1 % carboxymethyl cellulose (C.M.C) and administered orally.

**Experimental Procedure**

Animals, after acclimatization (6-7 days) in the animal quarters, were divided into five groups of six animals each with three males and three females in each group and treated in the following way:

Group I – served as "Normal Control" and received olive oil (1 ml/kg, i.p.) daily for 7 days.

Group II – served as "Toxicant Control" and received 1:1 (v/v) mixture of $CCl_4$ and olive oil (1 ml/kg, i.p.) daily for 7 days.

Group III - termed as "MUSE 250" and received MUSE (250 mg/kg, p.o.) daily for 10 days and a 1:1 (v/v) mixture of $CCl_4$ and olive oil (1 ml/kg, i.p.) for 7 days from day 4 to day 10.

Group IV - termed as "MUSE 500" and received MUSE (500 mg/kg, p.o.) daily for 10 days and a 1:1 (v/v) mixture of $CCl_4$ and olive oil (1 ml/kg, i.p.) for 7 days from day 4 to day 10.

Group V termed as "Reference Standard" and received silymarin (100 mg/kg, p.o.) for 10 days and a 1:1 (v/v) mixture of $CCl_4$ and olive oil (1 ml/kg, i.p.) for 7 days from day 4 to day 10.

Rats were sacrificed 24 h after the last injection by cervical dislocation and blood was collected by cardiac puncture. The collected blood was allowed to coagulate at Room Temperature for 30 min and serum was separated by centrifugation at 2500 rpm for 15 min. The separated serum was analyzed for the activities of marker enzymes [Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP)], bilirubin, albumin (Alb) and total proteins (TP). The livers were dissected immediately, washed with ice-cold saline and divided into two equal parts. One part was used to prepare a 10 % (w/v) homogenate in 1.15 % KCl solution. An aliquot was used for the determination of lipid peroxidation (LPO). The homogenates were centrifuged at 7,000 x g for 10 min at 4°C, and the supernatants were used for the assays of GSH level and SOD, CAT, GPx and GR activity. The remaining part of the liver was fixed in 10 % (w/v) buffered formalin and used for histological studies.

**Marker Enzyme assays**

The marker enzymes AST, ALT and ALP were assayed in serum using standard kits supplied from Span Diagnostics (Surat, India). The results were expressed as IU/L for AST, ALT and KA units for ALP.

**Protein estimation**

The levels of TP and Alb were determined in the serum of experimental animals by using the Lowry *et al*.,[13] method and the bromocresol green method as described by Webster *et al*.,[14], respectively. Kits purchased from Ranbaxy Laboratories, Delhi, India, were used for the estimation of Alb.

**Bilirubin estimation**

Levels of bilirubin were determined in serum by the method of Malloy and Evelyn,[15] using kits supplied by Span Diagnostics Ltd (Mumbai, India). The results were expressed as mg/100 ml for bilirubin.

**Lipid peroxidation**

The quantitative estimation of LPO was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in the liver using the method of Ohkawa *et al*.,[16]. The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as nmol of MDA/mg protein using molar extinction coefficient of the chromophore ($1.56 \times 10^{-5}$/M/cm) and 1,1,3,3-tetraethoxypropane as standard.

**Glutathione estimation**

GSH level was estimated in the liver homogenate using DTNB by the method of Ellman, [17]. The absorbance was read at 412 nm and the results were expressed as µmol/g of wet liver.

**Antioxidant enzyme assays in liver homogenate**

SOD was assayed by the method of Sun and Zigman,[18] in which the activity of SOD was inversely proportional to the concentration of its oxidation product adrenochrome, which was measured spectrophotometrically at 320 nm. 1

unit of SOD activity is defined as enzyme concentration required to inhibit the rate of autooxidation of epinephrine by 50 % in 1 min at pH 10.

CAT was estimated by the method of Clairborne,[9] which is a quantitative spectroscopic method developed for following the breakdown of $H_2O_2$ at 240 nm in unit time for routine studies of CAT kinetics.

GPx estimation was carried out using the method of Rotruck *et al*.,[20] which makes use of the following reaction:

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$
(oxidized glutathione)

GPx in the tissue homogenate oxidizes glutathione and simultaneously $H_2O_2$ is reduced to water. This reaction is arrested at 10 min using trichloroacetic acid and the remaining glutathione is reacted with DTNB solution to result in a coloured compound, which is measured spectrophotometrically at 420 nm.GR activity was determined by using the method of Mohandas *et al*.,[21] in which the following reaction is implicated:

$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2GSH$$

In the presence of GR, oxidized glutathione undergoes reduction and simultaneously, NADPH is oxidized to $NADP^+$. Enzyme activity is quantified at Room Temperature by spectrophotometrically measuring the disappearance of NADPH/min at 340 nm.

**Histopathological Studies**

The parts of the livers which were stored in 10 % (w/v) buffered formalin were embedded in paraffin, sections cut at 5 µm and stained with hematoxylin and eosin. These sections were examined under a light microscope for histoarchitectural changes.

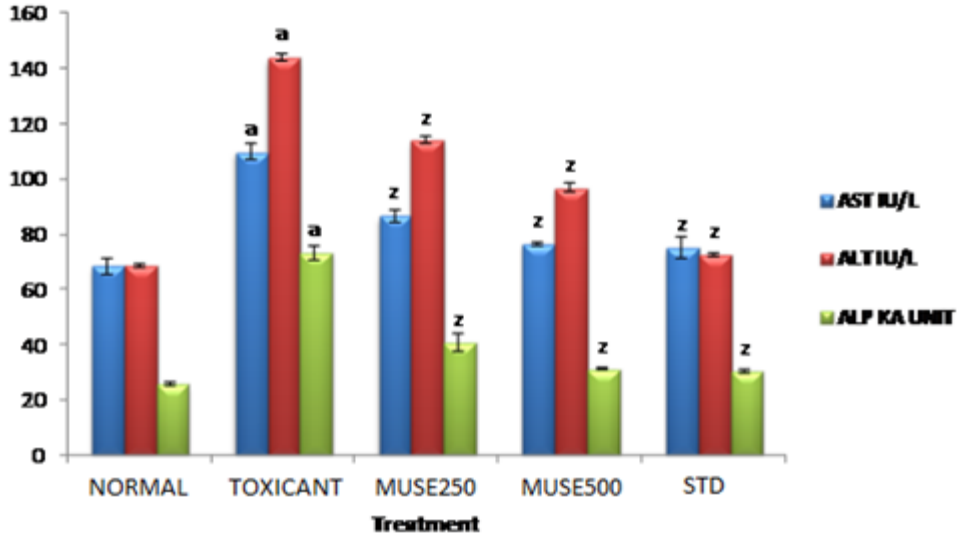
**Statistical analysis**

The results of hepatoprotective and antioxidant activities are expressed as mean ± SEM. Results were statistically analyzed using one-way ANOVA, followed by the Tukey-Kramer post test for individual comparisons. $P<0.05$ was considered to be significant.

**Results**

***Biochemical parameters***

The effect of MUSE on serum marker enzymes (AST, ALT and ALP) is shown in Figure 1. Significant elevation in AST, ALT and ALP activities was observed in the $CCl_4$ treated group of rats when compared with the Normal Control rats. Treatment of MUSE 250, MUSE 500 and




Values are mean ± SEM; $N = 6$ in each group; $P$ values: [a] < 0.001 when Toxicant Control compared with Normal Control; [z] < 0.001 when Experimental groups compared with Toxicant Control

**Figure 1.** Effect of Macrotyloma uniflorum seed extract **(**MUSE) on serum marker enzymes in $CCl_4$ –intoxicated rats

silymarin to $CCl_4$ intoxicated rats attenuated significantly ($p<0.001$) the $CCl_4$ elevated activities of these enzymes.

Figure 2 summarizes the effect of MUSE on serum proteins (TP and Alb) and serum bilirubin. In the $CCl_4$ treated rats a significant depletion in Alb and TP and a significant elevation in bilirubin levels was observed. Treatment with MUSE 250, MUSE 500 and silymarin followed by $CCl_4$ injection produced a significant elevation ($p<0.05$, $p<0.001$ and $p<0.001$ respectively) in the $CCl_4$-depleted Alb level; a significant increase ($p<0.001$) in the $CCl_4$-depleted TP level and a significant attenuation ($p<0.05$, $p<0.001$ and $p<0.001$ respectively) of the $CCl_4$elevated bilirubin level.

The effects of MUSE on MDA, GSH, SOD, CAT, GPx and GR are shown in Table 1. The free radical formation resulting in lipid peroxidation was measured in terms of MDA produced. There was a significant increase ($p<0.001$) in the MDA content in the liver homogenate in the $CCl_4$ treated group of rats when compared with the Normal Control rats. Treatment of MUSE 250, MUSE 500 and silymarin to $CCl_4$ intoxicated rats attenuated significantly ($p<0.001$) the increased formation of MDA due to $CCl_4$.

Significant depletion in GSH levels ($p<0.001$) was observed in the $CCl_4$ treated group of rats when compared with the Normal Control group of rats. Treatment with MUSE 500 and silymarin elevated significantly ($p<0.05$ and $p<0.001$ respectively) the GSH levels depleted by $CCl_4$.

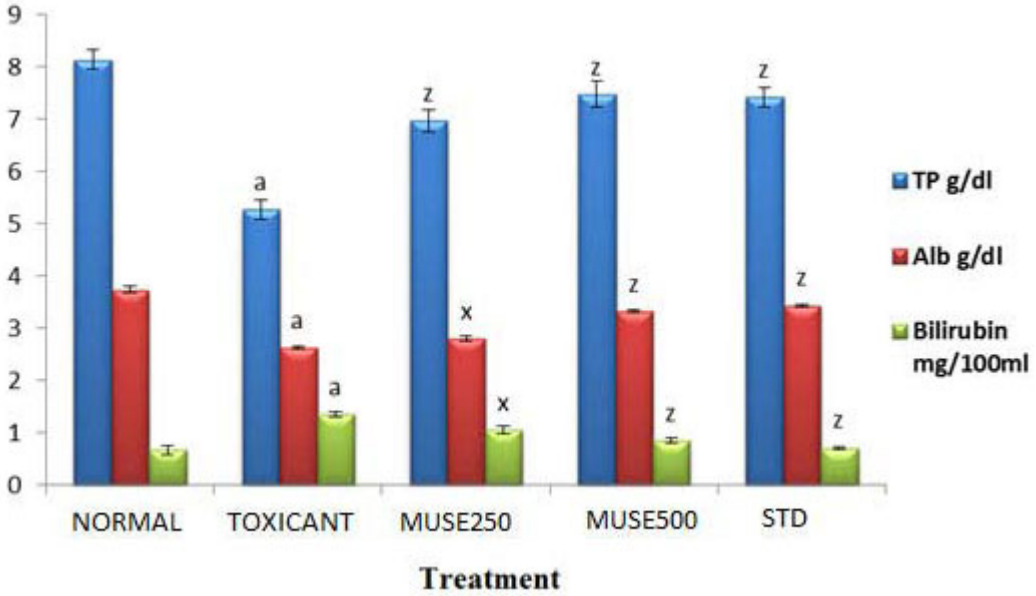
Liver SOD and CAT activities were examined to be strikingly lower ($p<0.001$) in $CCl_4$ treated rats when compared with normal rats. Treatment with MUSE 250, MUSE 500 and silymarin significantly ($p<0.001$) increased the $CCl_4$ lowered SOD and CAT activities.

GPx activity was depleted significantly ($p<0.001$) by $CCl_4$ treatment. Treatment with MUSE 500 and silymarin restored significantly ($p<0.001$) the $CCl_4$ depleted GPx activity.

Livers of rats treated with $CCl_4$ showed a significant reduction ($p<0.001$) in GR activity. MUSE 250 and MUSE 500 induced a significant ($p<0.001$) increase in the $CCl_4$ activity reduced by GR; the GR activity now being comparable with that exhibited by silymarin treatment.

**Histopathological studies**

The livers of animals in the Normal Control group showed normal cellular architecture with distinct hepatic cells with portal triad and compact arrangement with prominent nuclei and




Values are mean ± SEM; $N = 6$ in each group; $P$ values: $^{a} < 0.001$ when Toxicant Control compared with Normal Control; $^{x} < 0.05$, $^{z} < 0.001$ when Experimental groups compared with Toxicant Control

**Figure 2.** Effect Macrotyloma uniflorum seed extract **(**MUSE) on serum bilirubin, albumin and total proteins in $CCl_4$ –intoxicated rats

**Table 1. Effect of *Macrotyloma uniflorum* seed extract (MUSE) on liver TBARS, GSH, SOD, CAT, GPx and GR in $CCl_4$ intoxicated rats**

| Biochemical Parameters | Group I Normal Control Olive oil (1 ml/kg) | Group II Toxicant Control $CCl_4$ (1 ml/kg) | Group IV MUSE (250 mg/kg) | Group III MUSE (500 mg/kg) | Group V Silymarin (100 mg/kg) |
|---|---|---|---|---|---|
| TBARS (nmol MDA/min/mg protein) | 3.87±0.01 | 11.15±0.32[a] | 6.31±0.01[z] | 4.88±0.06[z] | 4.72±0.0007[z] |
| GSH (µmol/g of wet liver) | 1.89±0.10 | 0.85±0.16[a] | 1.17±0.18 | 1.55±0.19[x] | 1.67±0.10[z] |
| SOD (U/mg protein) | 1.53±0.006 | 0.54±0.003[a] | 1.03±0.01[z] | 1.33±0.003[z] | 1.40±0.001[z] |
| CAT (U/mg protein) | 3.27±0.09 | 1.26±0.008[a] | 1.94±0.05[z] | 2.78±0.03[z] | 2.87±0.04[z] |
| GPx (U/mg protein) | 8.56±0.21 | 4.22±0.70[a] | 5.37±0.61 | 7.84±0.29 [z] | 8.02±0.43 [z] |
| GR (U/mg protein) | 597.02±8.41 | 180.86±24.53[a] | 356.34±16.43[z] | 498.19±10.68[z] | 526.33±2.03[z] |

Values are mean ± SEM; $N = 6$ in each group

*P* values: [a] < 0.001 when Toxicant Control compared with Normal Control.

[x] < 0.05, [z] < 0.001 when Experimental groups compared with Toxicant Control

1 unit of CAT = µmol $H_2O_2$ consumed / min / mg protein

1 unit of GPx = µg GSH utilized / min / mg protein

1 unit of GR = nmol NADPH oxidized / min / mg protein

sinusoidal spaces (Fig.3a).

The livers of $CCl_4$ treated animals showed marked macrovesicular fatty degeneration diffusely throughout the liver with focal centrilobular necrosis of hepatocytes. In addition, diffuse minimal lymphocytic infiltration was also seen (Figure 3b).

Compared to the $CCl_4$ group of animals, the silymarin treated animals showed minimal degenerative changes in the liver. There was minimal centrilobular fatty and granular degeneration of hepatocytes and absence of necrosis and leukocytic infiltration (Figur 3c).

Lesions in the livers of the MUSE 250 mg/kg treated animals showed mild degree of macrovesicular fatty and granular degeneration around the central vein (Figure 3d).

The liver of animals in the MUSE 500 mg/kg treatment group showed minimal centrilobular fatty degeneration comparable to the standard silymarin treatment group (Figure 3e).

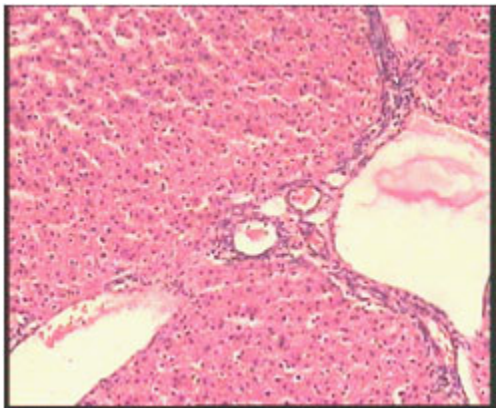


**Figure 3a.** Haematoxylin and eosin staining of liver of normal rat 10 X 10x = 100x

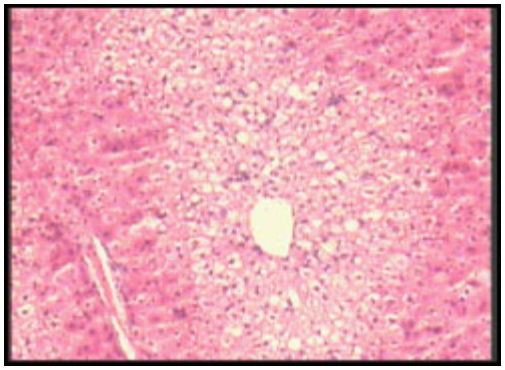


**Figure 3c.** Haematoxylin and eosin staining of liver of rat treated with carbon tetrachloride and silymarin (100mg/kg) 10 X 10x = 100x

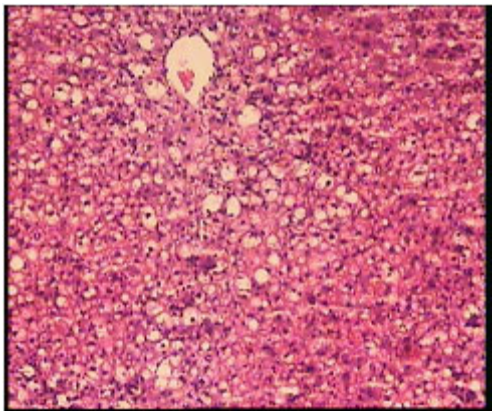


**Figure 3b.** Haematoxylin and eosin staining of liver of carbon tetrachloride treated rat 10 X 10x = 100x

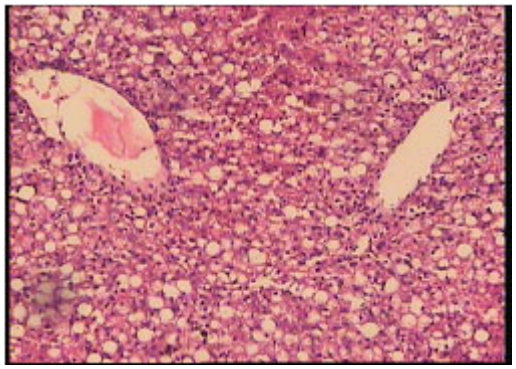


**Figure 3d.** Haematoxylin and eosin staining of liver of rat treated with carbon tetrachloride and MUSE ( 250 mg/kg ) 10 X 10x = 100x

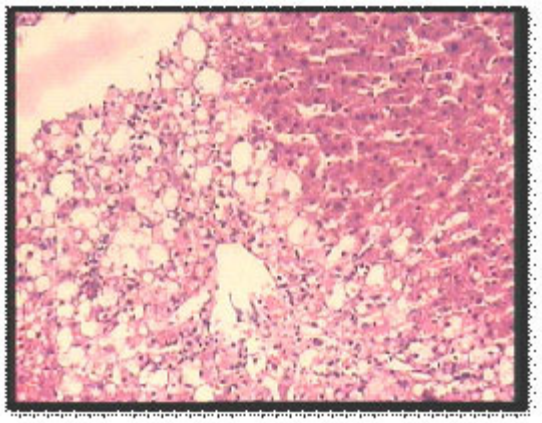


**Figure 3e.** Haematoxylin and eosin staining of liver of rat treated with carbon tetrachloride and MUSE ( 500 mg/kg) 10 X 10x = 100x

The histopathological examination of the livers of the treatment groups showed clear signs of retrieval from $CCl_4$ toxicity and provided a supportive evidence for the biochemical analysis. It was also evident that MUSE 500 showed better hepatoprotection than MUSE 250.

**Discussion**

$CCl_4$ is one of the most commonly used hepatotoxins in experimental hepatopathy. It is biotransformed by Cytochrome P-450 (CYP 2E1) to its active metabolite, the trichloromethyl ($CCl_3^{\cdot}$) radical which readily reacts with oxygen to form a trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$). These free radicals trigger cell damage through two mechanisms viz., i) covalent bonding to cellular macromolecules and ii) peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn yield products like MDA, which cause loss of integrity of cell membranes and damage to hepatic tissue [22].

Silymarin is a popular hepatoprotective used routinely as a reference standard in liver studies, especially for plant derived drugs. It is a mixture of three flavonolignans - silybin, silidianin, and silychristine extracted from the seeds of 'milk thistle' (*Silybum marianum*) [23]. Silymarin is used for the treatment of numerous liver disorders characterised by degenerative necrosis and functional impairment such as cirrhosis and alcoholic liver diseases. The hepatoprotection provided by silymarin appears to rest on four properties viz., a) its activity against lipid peroxi--dation as a result of free radical scavenging and the ability to increase the cellular content of GSH, b) its ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage, c) its capacity to regulate nuclear expression by means of a steroid-like effect and d) its ability to inhibit the trans-formation of stellate hepatocytes into myofibro-blasts, which are responsible for the deposition of collagen fibres leading to cirrhosis [24].

Evaluation of liver function can be done by estimating the activity of some marker enzymes such as AST, ALT and ALP which are originally present in high concentrations in the cytoplasm of the liver. When there is hepatic injury, these enzymes leak into the blood stream in conformity with the extent of liver damage [25]. The elevation in activities of these enzymes in the serum of $CCl_4$ treated rats in the present study was due to the extensive liver damage caused by $CCl_4$. Treatment with MUSE as well as the reference drug silymarin significantly reduced the elevation in the marker enzyme activities.

Total protein and albumin estimations are routinely performed to evaluate the toxicological nature of various chemicals. There was a marked depletion in the TP and Alb content in the serum of all rats after $CCl_4$ administration. MUSE treatment increased the levels of total proteins and albumin in the serum, which indicates its hepatoprotective activity. Stimulation of protein synthesis especially albumin has been advanced as a contributory hepatoprotective mechanism that accelerates the regeneration process and the production of hepatocytes [26]. The ability of MUSE to restore total protein and albumin levels may be due to the non-toxic antioxidant constituents present in the extract which aid liver cell regen-eration.

Bilirubin is a reddish-yellow pigment formed by the breakdown of hemoglobin in worn-out red blood cells. Normally, the liver conjugates bilirubin and it is excreted in bile and passes through the duodenum to be excreted. Bilirubin levels in the blood can be elevated due to over-production, decreased uptake by the liver, decrea-sed conjugation, decreased secretion from the liver or blockage of the bile ducts which happens in liver damage [27]. An increase in serum bilirubin level was observed in rats on $CCl_4$ administration. Treatment with MUSE attenuated significantly the $CCl_4$ elevated level of bilirubin by its cyto-protective action and may be also due to an inhibitory effect on cytochrome P-450.

An elevation in the levels of MDA, a LPO marker in $CCl_4$ treated rat livers was observed in the present study. Oral administration of MUSE decreased the lipid peroxidation of the cellular membrane suggesting a protective effect. The increase in MDA levels in the livers of $CCl_4$ treated rats suggests enhanced peroxidation

leading to tissue damage and failure of the antioxidant defense mechanisms to prevent the formation of excessive free radicals [28]. Treatment with MUSE significantly reversed these changes. Hence, it is likely that the mechanism of hepatoprotection of MUSE is due to its free radical scavenging activity.

Reduced glutathione, one of the most abundant non-enzymatic antioxidant bio-molecules present in the tissues is an important determinant of tissue susceptibility to oxidative damage [29]. It removes reactive oxygen species (ROS) such as hydroxyl radicals ($OH^{\cdot}$), superoxide radicals ($O_2^{\cdot}$) and alkoxy radicals, maintains integrity of membrane protein thiols and acts as a substrate for GPx and glutathione *S*-transferase (GST) [30]. Reduction of hepatic GSH level in $CCl_4$ induced liver toxicity occurs due to its increased utilization for augmenting the activities of GPx and GST [31]. In the present study, the decreased GSH level was significantly elevated in rats treated with MUSE 500 and silymarin. This increase may be due to *de novo* GSH synthesis, GSH regeneration or by stimulation of GR activity.

Free radical scavenging enzymes such as SOD, CAT & GPx are known to be the first line cellular defense enzymes against oxidative damage, disposing $O_2$ & $H_2O_2$ before their interaction to form the more harmful hydroxyl ($OH^{\cdot}$) radical [32]. In the present study SOD activity decreased significantly in the $CCl_4$ treated group of animals, which might be due to an excessive formation of superoxide anions. These excessive superoxide anions might inactivate SOD and decrease its activity. In the absence of adequate SOD activity, superoxide anions are not dismuted into $H_2O_2$, which is the substrate for the $H_2O_2$ scavenging enzymes CAT & GPx. As a result, there is an inactivation of the $H_2O_2$ scavenging enzymes CAT and GPx, leading to a decrease in their activities. Administration of MUSE to $CCl_4$ in-toxicated rats effectively prevented the depletion of SOD, CAT & GPx activities, which can be correlated to the scavenging of free radicals by MUSE, resulting in protection of these enzymes.

GR is an antioxidant enzyme involved in the reduction of GSSG (an end product of GPx reaction) to GSH. In $CCl_4$ treated rats, there was a marked depletion of GPx activity, leading to reduced availability of substrate for GR, thereby decreasing the activity of GR. Oral treatment of MUSE to $CCl_4$ intoxicated rats restored the activity of GR, thus accelerating the conversion of GSSG to GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH.

The biochemical observations were supported by a histopathological examination of the rat livers. The hepatoprotective effect of MUSE may, thus be attributed to an underlying antioxidant activity, which prevents the process of initiation and progress of hepatocellular injury. The effects of MUSE were comparable with those of silymarin, a proven hepatoprotective. MUSE possesses strong antioxidant properties probably due to the presence of various phenolic com-pounds, flavonols and tannins.

## Conclusion

In conclusion, the hepatoprotective effect of MUSE in $CCl_4$ induced hepatotoxicity in rats appears to be related to its ability to inhibit lipid peroxidative processes and prevent GSH depletion. The present study, thus, aims to promote the use of this "poor man's pulse" in people's diets and establish it as a potent "functional food". Besides, it is cheap, packed with protein and low on fat. Further research may be carried out to isolate the active constituents of *Macrotyloma uniflorum* seeds which are responsible for its hepatoprotective activity.

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