# ETHANOLIC EXTRACT OF *MUSA SAPIENTUM* SPOTTED PEEL TREATMENT FOR BETTER AMELIORATES CARBON TETRACHLORIDE NEPHROTOXICITY

**Ajra Khan*[1], Pushpa Simaiya[2], Dr. Deepak Basedia[3], Dr. B. K. Dubey[4]**

[1]Technocrats Institute of Technology Pharmacy, Bhopal
[2]Associate Professor, Department of Pharmacology, Technocrats Institute of Technology Pharmacy, Bhopal
[3]Head of the department, Technocrats Institute of Technology Pharmacy, Bhopal
[4]Director, Technocrats Institute of Technology Pharmacy, Bhopal
Email: ajrakhan25@gmail.com[1]
Pushpasimaiya87@gmail.com[2]
deepakbasedia@gmail.com[3]
*Corresponding author
Ajra Khan
Master's scholar,Department of Pharmacology, Technocrats Institute of Technology Pharmacy, Technocrats Group Campus Anand Nagar, BHEL Bhopal-462021 Madhya Pradesh

***ABSTRACT***

*When kidney-specific detoxification and excretion are impaired because of endogenous or exogenous toxins damaging or destroying kidney function, this condition is known as Nephrotoxicity. Ethnomedicinal plants from the traditional system of medicine viz Ayurveda and Unani, which are acclaimed by the Ayurvedic and Unani physicians to have nephroprotective properties and commonly used to treat various renal disorders, have been extensively investigated for their significant nephroprotective effects. Musa sapientum var. sylvesteris (M. sapientum var. sylvesteris) is a valuable medicinal plant which belongs to the family Musaceae. The plant widely grows in Bangladesh. Banana is generally consumed as a dessert or cooked as vegetable or made into various confections. There are various types of species in Musa genus and their pharmacological studies have been studied. In present investigation the medicinal plants Musa sapientum peel was used to investigate for their protective ability of the kidney against CCl4- induced nephrotoxicity. In our studies simultaneous supplementation of EEMS to CCl4- treated rats was found to ameliorate the renal toxicity. Therefore, it is assumed that administration of EEMS act on ROS induced by CCl4.*

***KEY WORDS:****Nephrotoxicity, Musa sapientum, Phytochemical Invastigation, $CCl_4$,*

# INTRODUCTION

Plants have long been a significant source of pharmaceuticals and an abundant source of atypical pharmaceuticals. It has been discovered that 5-15% of the more than 200,000 varieties of indigenous plants that have been methodically examined for their therapeutic properties in various medicinal systems could be a very good source of new biologically active compounds and may be used as pharmacotherapeutic agents. Derivatives of herbal products and medicines made from herbal plants are very effective in the healthcare system because they are cost effective, bio-friendly, relatively safe and easily available mainly in developing countries. Herbal plants are important source of effective medicinal agents, for e.g. reserpine, noscapine, codeine, sennoside, guggul steroids, deserpidine, digitalis cardiac glycosides, vinica alkaloids, morphine, artemisin, guggulsterone and ginkgotoxin, that have been included directly or their synthetic analogues included into different herbal medicines (Kamboj[2]., 2000; Mukherjee[3]., 2010). India has abundance of herbs and has ability to supply medicinal plants for fulfilling its global requirements. In India, approximately 65% to 70% of contemporary medicines are obtained from herbal sources and plants are served as lead for most of synthetic drugs. It is estimated that only very few (around 6-7%) of plant species of all therapeutically important plants have been analysed chemically and a very small portion of it have been analysed pharmacologically (Sharma A [4]2008).

Seventy percent of Indians use herbs or herbal medications to improve their health. Plants/herbs have long been used as a source of pharmaceuticals for the treatment of various diseases in many traditional medical systems (Vaidya [5]2007). People in the twenty-first century are turning to herbal products and herbal medications to improve their health. People have been employing herbal remedies as medicinal agents for a long time (alone or in combination with other agents). According to World-Health-Organization research, a huge population globally uses herbal products for basic healthcare. Herbal medications are made up of entire herbs, herbal substances (such as different portions of plants), and active compounds derived from plants, as well as processed and completed herbal preparations. (Litsherand Goa [6]2014; Sen [7]2010).

Kidney is the primary organ of urinary system in human body; it purifies the blood by removing and excreting out wastes from the body through urine. Further, it also helps in maintaining the fluid homeostasis, electrolyte balance, blood pressure, regulation of the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs etc. Hence, it is one of the vital organs of the body. The kidneys filter approximately 180 L of blood each day, and as a result of this large volume, the kidneys are more often exposed to toxic blood constituents and are more susceptible to injury from a variety of sources.

Chronic exposure to drugs, occupational hazards or environmental toxins can lead to chronic renal diseases. Nephrotoxicity is the third most common problem of the renal system with an estimated lifetime risk of 8-15% in Europe and America, 20% in the Middle East and around 2-5% in Asia. Several therapeutic agents can adversely affect the kidney resulting in acute renal failure.

Antioxidants are compounds that could protect against the damage that unstable molecules called free radicals are doing to cells. Free radicals can kill the body's healthy cells, causing them to lose their structure and functionality. This results in cell disruption, aging, and

degenerative disorders such as cancer, cardiovascular disease, cataracts, immune system deterioration, and brain malfunction. Free radicals are electrically charged molecules with an unpaired electron, causing them to seek and capture electrons from other substances in order to neutralize themselves. Free radicals are known to be stabilized or deactivated by antioxidants before they attack cells.

When kidney-specific detoxification and excretion do not work adequately in response to exogenous or endogenous toxicant damage or loss of kidney function, this is known as Nephrotoxicity. Nephroprotective agents are substances that can reduce the effects of nephrotoxic compounds. Because of different complex chemical compounds, medicinal plants offer curative capabilities. Ethnomedicinal plants from the traditional system of medicine viz Ayurveda and Unani, which are acclaimed by the Ayurvedic and Unani physicians to have nephroprotective properties and commonly used to treat various renal disorders, have been extensively investigated for their significant nephroprotective effects.

*Musa sapientum* var. *sylvesteris* (*M. sapientum* var. sylvesteris) is a valuable medicinal plant that belongs to the family Musaceae. The plant widely grows in Bangladesh. Banana is commonly eaten as a dessert, cooked as a vegetable, or put into various confections. Aside from being a very nutritious delicacy, banana fruits and other components of the banana tree have a variety of purposes in medicine, fiber production, religious ceremonies, etc.

Various types of species exist in *the Musa* genus, and their pharmacological studies have been studied. In the present investigation, the medicinal plant *Musa sapientum* peel was used to investigate the protective ability of the kidney against CCl4- induced nephrotoxicity.

## MATERIAL AND METHODS

### Collection of plant material

The plants have been selected based on their availability and folk use of the plant. Every part of the plant, like bark, leaves, flowers, roots, fruits, and seeds, may contain active secondary metabolites. Fresh & healthy plant materials, free from diseases of Musa sapientum were collected from the local market of Bhopal (M.P.) in the month of March 2022.

### Extraction procedure

Extraction is essential in phytochemical processing to find the bioactive secondary metabolite from plant materials. Selecting a suitable extraction technique is also necessary for the standardization of herbal products. Extraction is used in the removal of desirable soluble constituents and exclusion of those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. The following procedure will be adopted to prepare extract from the shade-dried material (Ansari [8], 2001).

### Defatting of plant material

In a maceration process, 78.18 grams of Musa sapientum shade-dried plant material were coarsely crushed and extracted with petroleum ether at 60–80 °C. The extraction process was maintained until the material had been sufficiently defatted.

**Extraction by maceration process**

Defatted plant materials of Musa sapientum were exhaustively extracted with hydroalcoholic solvent (methanol: aqueous: 70:30v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated for the dried extracts (Mukherjee [9], 2007).

**Determination of percentage yield**

The extraction yield is evaluated of the solvent's efficiency in extracting bioactive components from the selected natural plant samples. It was defined as the quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, the yield of the plant extracts obtained was calculated in grams and then converted into a percentage. The following formula was adopted to determine the percentage yield of selected plant materials. The percentage yield of each extract were calculated by using the following formula:

$$\textbf{Percentage Yield} = \frac{\boldsymbol{Weight\ of\ Extract}}{\boldsymbol{Weight\ of\ Powder\ drug\ taken}} \boldsymbol{x}\ \mathbf{100}$$

**Phytochemical Screening**

Medicinal plants are resources of traditional medicines, and many modern drugs are produced indirectly from plants. Phytochemical constituents are of two types primary bioactive constituents (chlorophyll, proteins, amino acids, sugar, etc.) and secondary bioactive constituents, including (alkaloids, terpenoids, phenols, flavonoids, etc.). Phytochemical examinations were carried out for all the extracts per standard methods (Khandelwal [10], 2005).

## QUANTITATIVE ESTIMATION OF BIOACTIVE COMPOUNDS

**Total phenolic content estimation**

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid were dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract were dissolved in 10 ml methanol and a filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard were mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for color development. A UV spectrophotometer was used to measure absorbance at 765 nm. (Parkhe and Bharti [12], 2019).

**Total flavonoids content estimation**

The determination of total flavonoid content was based on the aluminum chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and a filter. Three ml (1mg/ml) of this extract was used to estimate flavonoids. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Parkhe and Bharti [12], 2019).

## *In Vivo*Nephroprotective Activity

### Animals

This investigation used Wistar Rats (180-220 g). They were fed a regular meal, given tap water and labium, and subjected to a 12-hour light/dark cycle. Before the studies, the animals were acclimatized to the laboratory environment. The Institutional Animal Ethics Committee approved the experimental protocol. The animals were cared for following the standards of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. The Institutional Animal Ethics Committee approved the protocol for the experiment.

### Acute Oral Toxicity Study

Adult Wistar Rats of either sex weighing between (180-220 g) were fasted overnight and used for acute toxicology testing in accordance with the Organization for Economic Cooperation and Development (OECD 423) standard. Four groups of mice of both sexes fasted overnight. The first control group of mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water. In comparison, the other three groups received ethanolic extracts of Musa sapientum (EEMS) suspended in 0.5% CMC at 200, 600, and 2000 mg/kg doses. Animals were observed closely for the first 4 hours for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently, observations were made at regular intervals for 24 h. The animals were under further investigation for up to 14 days, and no mortality was reported within the study period.

### Experimental Protocol

Animals after acclimatization (7 days) in the animal quarters were fasted overnight and randomly divided into five groups of 6 animals each and treated in the following way:

Group I served as Control.

Group II Rats received ethanolic extracts of Musa sapientum (EEMS) 150 mg/kg daily in drinking water orally for eight weeks.

Group III Rats were injected with carbon tetrachloride (2mL/Kg, i.p.) once weekly for 12 weeks (Sohn [13]., 1991).

Group IV Rats received ethanolic extracts of Musa sapientum (EEMS) 150 mg/kg daily in drinking water for eight weeks; also, the rats were injected with $CCl_4$(2mL/Kg b.wt., i.p.) once weekly for eight weeks.

Group V Rats received Silymarin (50 mg/kg wt. p.o. for eight weeks); the rats were injected with carbon tetrachloride (2mL/Kg b.wt. i.p.) once weekly for eight weeks.

24 hours following the final dose, the animals were slaughtered. The blood was drawn through cervical dislocation and clotted at room temperature for 30 minutes. Centrifugation was used to separate the serum for 15 minutes at 4 °C at 3000 rpm and used to estimate the marker kidney function parameters plasma protein, urea, and creatinine. The kidney was dissected immediately and washed with ice-cold saline, and 10% homogenates in 50 mM Tris-HCl and 300 mM sucrose were prepared. Centrifuging the homogenates at 5000 g for 10 min at 4°C and the supernatants were used for the assay of GSH, SOD, CAT, GPx, GST, and GR.

**Kidney Index**

At the end of the experimental period, each rat was weighed. The left kidney was then removed and weighed. Finally, the kidney index was calculated by dividing left kidney weight by body weight and multiplying it by 100.

**Biochemical Analysis**

Blood samples were collected by the retro-orbital method using heparin-coated capillaries. The serum was separated by centrifugation for 5min at 1000×g and stored at −20°C until analysis. Serum samples were used to determine alkaline phosphate, Creatinine, blood urea nitrogen, uric acid, total proteins, cholesterol, and albumin level. All estimations were performed using a diagnostic kit (Gupta Diagnostic center, Bhopal) as per the standard methods.

**Antioxidant Enzyme Assays**

Using a Potter-Elvehjem homogenizer at 4 °C and 0.15 M KCl, a 10% kidney homogenate was prepared. The debris was removed from the homogenate by centrifuging it (12,000 rpm for 45 min at 0-4 °C) in a Remi (C24-BL) cooling centrifuge. The supernatant was then used for enzyme assays.

**Glutathione Peroxidase (Gpx) Assay**

Glutathione peroxidase activity was estimated as described by Rotruck [14]. (1973) and Ellman's[15] (1959). Briefly, 0.5 ml 0.4 M buffer (pH 7.0), 0.2 ml enzyme source (kidney homogenate), 0.2 ml 2mM GSH, 0.1 ml 0.2 mM $H_2O_2$ were added and incubated at room temperature for 10 min along with a control tube containing all reagents except enzyme source. The reaction was arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min, and the GSH content in 0.5 ml of supernatant was estimated. The activity was expressed as µg of GSH consumed/min/mg protein.

**Glutathione Reductase (GR) Assay**

Glutathione reductase activity was estimated by Pinto and Bartley[16] (1969). To 0.5 ml of 0.25 M potassium phosphate buffer (pH 7.4), 0.1 ml of 25 mM EDTA, 0.1 ml of 1 mM NADPH, 0.96 ml of distilled water, and 0.1 ml of enzyme source (kidney homogenate). The reaction was initiated by adding 0.024 ml GSSG (50 mM). The change in absorbance was recorded at 1-min intervals at 340 nm for 5 min. The specific activity is expressed as µmol of NADPH oxidized/min/mg protein using an extinction coefficient for NADPH of 6.22 cm-1 mmol-1.

**Glutathione-S-Transferase (GST) Assay**

The glutathione-S-transferase activity was estimated as described by Habig[17] (1974). To 1.7 ml of 0.14 M buffer (pH 6.5), 0.2 ml 30 mM GSH, and 0.04 ml enzyme source (kidney homogenate). The reaction was initiated by 0.06 ml 0.01 M 1-chloro-2, 4-dinitrobenzene (CDNB). The activity was determined using a 9.6 mM-1 CDNB-GSH conjugate extinction

coefficient and expressed as the moles of CDNB-GSH conjugate produced per minute per mg of protein.

**Catalase (CAT) Assay**

Catalase activity was estimated by the Beers and Sizer[18] (1952) method. The assay system contained 1.9 ml 0.05 M buffer (pH 7.0) and 1.0 ml 0.059 M H2O2. The reaction was initiated by adding a 0.1 ml enzyme source (kidney homogenate). The decrease in absorbance was monitored at 1 min intervals for 5 min at 240 nm, and activity was expressed as n moles of $H_2O_2$ decomposed/ min/mg protein.

**Superoxide Dismutase (SOD) Assay**

Superoxide dismutase activity was estimated by the method of Marklund and Marklund[19] (1974) adopted as follows by Soon and Tan[20] (2002): To 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source (kidney homogenate) and 0.86 ml of distilled water. The reaction was initiated with 0.02 ml of 10 mM pyrogallol, and the change in absorbance was monitored at 420 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50 % in a standard assay system of 3 ml. The specific activity was expressed as units/min/mg protein. Lowry[21] (1951) estimated the protein concentration of the supernatant using the crystalline BSA standard.

**Statistical Analysis**

The results were expressed as mean ± S.D. The results were analyzed using the DMR test (Duncan's Multiple Range Test). $P<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

The present study revealed an ameliorative effect of EEMS on CCl4-induced renal toxicity in rats. The pathogenesis of kidneys is a crucial public health problem. It is well known that the kidneys play a pivotal role in the regulation of various chemicals. Administration of CCl4 causes Nephrotoxicity as indicated by an elevation in urine and serum level of urea, Creatinine, and urobilinogen while it decreases the Creatinine clearance. These pathological changes signify the potential damage to liver and kidney cells induced by CCl4 treatment (Ogeturk[22], 2005).

The present study shows that elevation in plasma urea and creatinine levels can be attributed to the damage to nephron structural integrity (Khan and Siddique[24], 2012). In addition, a decrease in the total plasma proteins and albumin concentrations in CCl4-treated rats might have resulted from remarkable leakage due to glomerular and tubular hyper-cellularity.EEMS treatment significantly improved the concentrations of total proteins and albumin in plasma, while a significant recovery was noticed in the levels of urea and Creatinine.

Earlier studies have also shown that extract treatment decreased serum creatinine and urea concentrations in dose-dependent cyclosporine-induced renal injury in rats. This effect may be related to the antioxidant properties of curcumin since it has been found that ROS may be involved in the impairment of the glomerular filtration rate. Superoxide dismutase and catalase are extremely effective antioxidants responsible for the catalytic dismutation of

highly reactive toxic superoxide radicals to H2O2 and for the catalytic decomposition of H2O2 to oxygen and water, respectively.

CCl4-induced oxidative stress in renal tissues led to an accumulation of superoxides and hydrogen peroxides. This study evidenced a decline in GPx, GR, GST, CAT, and SOD activities in the kidney tissue. These results agreed with earlier findings (Khan and Siddique[24], 2012). Curcumin treatment significantly improved the antioxidant enzyme activity in the kidney tissue.

**Table 1: Results of percentage yield of extract of *Musa sapientum***

| S. No. | Hydroalcoholic extract | Percentage yield (w/w) |
|---|---|---|
| 1 | *Musa sapientum* | 10.4% |

**Table 2: Result of phytochemical screening of extract of *Musa sapientum***

| S. No. | Constituents | Hydroalcoholic extract |
|---|---|---|
| 1. | Alkaloids<br>A) Hager's Test | <br>-Ve |
| 2. | Glycosides<br>A) Legal's Test: | <br>-Ve |
| 3. | Flavonoids<br>A) Lead acetate Test:<br>B) Alkaline Reagent Test: | <br>+Ve<br>+Ve |
| 4. | Saponins<br>A) Froth Test: | <br>+Ve |
| 5. | Phenolics<br>A) Ferric Chloride Test: | <br>+Ve |
| 6. | Proteins<br>A) Xanthoproteic Test: | <br>+Ve |
| 7. | Carbohydrate<br>A) Fehling's Test: | <br>+Ve |
| 8. | Diterpenes<br>A) Copper acetate Test: | <br>+Ve |

**Table 3: Estimation of total phenolic and flavonoids content of*Musa sapientum***

| S. No. | Hydroalcoholic extract | Total phenol content | Total flavonoids content |
|---|---|---|---|
| 1. | *Musa sapientum* | 0.251 mg/100mg | 0.563 mg/100mg |

**Table 4: Effect of EEMS on relative kidney weight of different treated groups**

| Name of the Experimental group | Drug /Dose | Relative Kidney (wt/100g bw) |
|---|---|---|
| Group I: Normal control | Normal saline | 0.631 ±0.122 |
| Group II: EEMS control | 150 mg/kg,p.o | 0.529 ±0.0823 |
| Group III: $CCl_4$ only | (2mL/Kg, i.p.) once weekly | 0.895 ±0.02 |
| Group IV: $CCl_4$ + EEMS | (2mL/Kg, i.p.) once weekly +150 mg/kg,p.o daily | 0.752 ±0.0445 |
| Group V: $CCl_4$ + Silymarin | 2mL/Kg, i.p.) once weekly + 50 mg/kg,p.o daily | 0.707 ±0.065 |

Values are mean ± S.D. (n=6) ; Values with different superscripts within a column are significantly different at ($P<0.05$).

**Table 5: Effect of EEMS on plasma protein of different treated groups**

| Name of the Experimental group | Drug /Dose | Total protein (g/dL) |
|---|---|---|
| Group I: Normal control | Normal saline | 9.565 ±0.335 |
| Group II: EEMS control | 150 mg/kg,p.o | 9.3817 ±0.3 |
| Group III: $CCl_4$ only | (2mL/Kg, i.p.) once weekly | 6.09 ±0.1719 |
| Group IV: $CCl_4$ + EEMS | (2mL/Kg, i.p.) once weekly +150 mg/kg,p.o daily | 8.565 ±0.2843 |
| Group V: $CCl_4$ + Silymarin | 2mL/Kg, i.p.) once weekly + 50 mg/kg,p.o daily | 9.1 ±0.1243 |

Values are mean ± S.D. (n=6) ; Values with different superscripts within a column are significantly different at ($P<0.05$).

**Table 6: Effect of EEMS on albumin of different treated groups**

| Name of the Experimental group | Drug /Dose | Albumin (g/dL) |
|---|---|---|
| Group I: Normal control | Normal saline | 5.6 ±0.287 |
| Group II: EEMS control | 150 mg/kg,p.o | 5.531 ±0.09 |
| Group III: $CCl_4$ only | (2mL/Kg, i.p.) once weekly | 3.411 ±0.2724 |
| Group IV: $CCl_4$ + EEMS | (2mL/Kg, i.p.) once weekly +150 mg/kg,p.o daily | 4.37 ±0.2277 |
| Group V: $CCl_4$ + Silymarin | 2mL/Kg, i.p.) once weekly + 50 mg/kg,p.o daily | 4.97 ±0.074 |

Values are mean ± S.D. (n=6) ; Values with different superscripts within a column are significantly different at ($P<0.05$).

**Table 7: Effect of EEMS on plasma urea of different treated groups**

| Name of the Experimental group | Drug /Dose | Urea (mg %) |
|---|---|---|
| Group I: Normal control | Normal saline | 29.15 ±0.2832 |
| Group II: EEMS control | 150 mg/kg,p.o | 29.1617 ±0.2 |
| Group III: $CCl_4$ only | (2mL/Kg, i.p.) once weekly | 66.7 ±0.6316 |
| Group IV: $CCl_4$ + EEMS | (2mL/Kg, i.p.) once weekly +150 mg/kg,p.o daily | 37.99 ±0.695 |
| Group V: $CCl_4$ + Silymarin | 2mL/Kg, i.p.) once weekly + 50 mg/kg,p.o daily | 36.16 ±0.4479 |

Values are mean ± S.D. (n=6) (EEMS - EEMS); Values with different superscripts within a column are significantly different at (P<0.05)

**Table 8: Effect of EEMS on plasma Creatinine of different treated groups**

| Name of the Experimental group | Drug /Dose | Creatinine (mg %) |
|---|---|---|
| Group I: Normal control | Normal saline | 0.6367 ±0.02 |
| Group II: EEMS control | 150 mg/kg,p.o | 0.6567 ±0.02 |
| Group III: $CCl_4$ only | (2mL/Kg, i.p.) once weekly | 1.6517 ±0.035 |
| Group IV: $CCl_4$ + EEMS | (2mL/Kg, i.p.) once weekly +150 mg/kg,p.o daily | 0.8765 ±0.0076 |
| Group V: $CCl_4$ + Silymarin | 2mL/Kg, i.p.) once weekly + 50 mg/kg,p.o daily | 0.7235 ±0.01 |

Values are mean ± S.D. (n=6) (EEMS - EEMS); Values with different superscripts within a column are significantly different at (P<0.05)

**Table 9: Effect of EEMS and vitamin E on antioxidant enzymes activities (µg or µmol/min/mg protein) in the kidney of different treated groups**

| Name of the parameter | Group I Vehicle control | Group II EEMS control | Group III $CCl_4$ | Group IV $CCl_4$+ EEMS | Group V $CCl_4$ + Silymarin |
|---|---|---|---|---|---|
| GPx (µg GSH/ min/mg protein) | 8.8 ±0.25 | 8.89 a±0.38 | 4.538±0.295 | 6.78 ±0.195 | 8.665 ±0.217 |
| GR (µM/ min/mg protein) | 1.677 ±0.025 | 1.653 ±0.02 | 0.72±0.004 | 1.1007±0.0503 | 1.376 ±0.019 |
| GST (µM/ min/mg | 0.0485 ±0.0013 | 0.0479 ±0.0013 | 0.0187 ±0.0002 | 0.0428 ±0.002 | 0.045 ±0.0008 |

| | | | | | |
|---|---|---|---|---|---|
| protein) | | | | | |
| CAT (µM $H_2O_2$/ min/mg protein) | 25.298 ±0.111 | 25.06 ±0.2349 | 12.36 ±0.281 | 20.354 ±0.29 | 22.416 ±0.2952 |
| SOD (U/min/mg protein) | 7.411 ±0.2994 | 7.188 ±0.16 | 2.9716±0.0587 | 5.6748 ±0.2258 | 6.3952 ±0.3431 |

Values are mean ± S.D. (n=6) (EEMS - EEMS); Values with different superscripts within a column are significantly different at P.

## CONCLUSION

This suggests that antioxidant potential of EEMS. More and more studies now established the ability of curcumin to mainly eliminate the hydroxyl radical (Reddy and Lokesh[24], 1994), superoxide radical (Sreejayan and Rao[25], 1996), singlet oxygen (Rao [26], 1995), nitrogen dioxide (Unnikrishnan and Rao [27], 1995) and NO (Sreejayan and Rao [25], 1997). It has also been demonstrated that EEMS inhibits the generation of the superoxide radical (Ruby [28]1995). In our studies simultaneous supplementation of EEMS to $CCl_4$- treated rats was found to ameliorate the renal toxicity. Therefore, it is assumed that administration of EEMS act on ROS induced by $CCl_4$.

## REFERENCES


1. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads, Pure Applied Chemistry 2005; 77: 7-24.
2. Kamboj V. Herbal medicine or modern medicine, Current Science 2000; 78: 35-39.
3. Mukherjee P K, Ponnusankar S and Venkatesh M. Ethno medicine in complementary therapeutics. In: Chattopadhyay D, editor. Ethanomedicine: A Source of Complementary Therapeutics. Trivandrum (India), Research Signpost 2010: 29-52.
4. Sharma A, Shanker C, Tyagi L K, Singh M. and Rao Ch V: Herbal medicine for market potential in India: an overview. Journal of Plant Sciences 2008; 1: 26-36.
5. Vaidya A D B. Devasagayam T P A. Current status of herbal drugs in India: an overview. Journal of Clinical Biochemistry and Nutrition 2007; 4: 1-11.
6. Litscher G. Gao S. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources, Evidence Based Complementary and Alternative Medicine 2014; 1-20.
7. Sen S, Chakraborty R, De B, Ganesh T, Raghavendra H G and Debnath S. Analgesic and inflammatory herbs: a potential source of modern medicine. International Journal of Pharmaceutical Science and Research 2010; 1: 32- 44.
8. Ansari HS. Essentials of Pharmacognosy, NiraliPrakashan, Pune, 2001; 591-592.
9. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
10. Khandelwal KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rdEdn: 2005; 15.
11. Kokate CK. Ed. Practical Pharmacognosy, 4thEdn., *Vallabh Prakashan*: 1994; 112:120.

12. Parkhe G, Bharti D. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora*Roxb. JDDT. 2019; 9(4-A):708-11.
13. Sohn, D.H., Yun, Y.P., Park, K.S., Veech, R.L., Song, B.J., 1991. Post-translational reduction of cytochrome P450IIE by CCl4, its substrate. BiochemBiophys Res Commun 179, 449-454.
14. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DC, Hoekstra WG. Selenium: biochemical roles as a component of glutathione peroxidase. Science 1973; 179: 588-90.
15. Ellman G. Tissue sulphhydril. Arch BiochemBiophys 1959; 82:70-7.
16. Pinto RE, Bartley W. The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic GSH oxidation in rat liver homogenates. Biochem J 1969; 112:109-15.
17. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 246:7130-9.
18. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of ydrogen peroxide by catalase. J Biol Chem 1952; 195:133-40.
19. Marklund SL, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47:469-74.
20. Soon YY, Tan BKH. Evaluation of the hypoglycemic and anti-oxidant activities of Morinda officinalis in streptozotocininduced diabetic rats. Singapore Med J 2002; 43:77-85.
21. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193:265-75.
22. Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M. Caffeic acid henethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J Ethnopharmacol 2005; 28:97:273-80.
23. Khan MR, Siddique F. Antioxidant effects of Citharexylum spinosum in CCl4 induced nephrotoxicity in rat. Exp Toxicol Pathol 2012; 64:349-55.
24. Reddy AC, Lokesh BR. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol Cell Biochem 1994; 137:1-8.
25. Sreejayan N, Rao MN. Free radical scavenging activity of curcuminoids. Arzneimittelforschung 1996; 46:169-71.
26. Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res 1995; 55:259-66.
27. Unnikrishnan MK, Rao MN. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. Mol Cell Biochem 1995; 146: 35-7.
28. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Antitumour and antioxidant activity of natural curcuminoids. Cancer Lett 1995; 94:79-83.
29. Gbemisola A. Adegoke, Samuel A. Onasanwo, O. David Eyarefe, Samuel B.Olaleye. Ameliorative effects of *Musa sapientum* peel extract on acetic acid-induced colitis in rats. The Journal of Basic & Applied Zoology. Volume 77, 2016, 49-55.