

Screening Of *Mansoa alliacea* Leaf Extracts Hepatoprotective Activity Against Carbon Tetrachloride-Induced Liver Damage Model

Srilakshmi Gunda¹, Dr.M.VijeyAanandhi^{2*}

¹Research Scholar, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India.

²Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India.

*Corresponding author Mail id: hodpchemistry@velsuniv.ac.in

ABSTRACT

Leaves of *Mansoa alliacea* Lam were extracted using Hexane and ethanol as solvents in a consecutive manner. Processed extracts were evaluated for hepatoprotective activity on Wistar albino rats using CCl₄ induced liver damage model. Hepatoprotective activity was assessed by comparing the control group with the standard group for serum enzyme levels such as S.G.P.T., S.G.O.T., T.B., and A.L.P. obtained from the test groups with the toxic group. It could be interpreted from the obtained results that the ethanolic extract-treated group showed highly significant activity; the hexane extract-treated group also has shown significant action but less than ethanolic extract. Histopathological studies supported the obtained results.

KEYWORDS *Mansoa alliacea*, Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Total bilirubin, Alkaline phosphatase.

Introduction:

The liver acts as the largest organ for the detoxification process. Helps in the removal of viruses, toxins, and other residues from the body. The liver is located between the portal and the general circulation, between the gastrointestinal tract and the heart¹. Multiple functions will be performed by the liver like nutrients storage, converting it to necessary form, and all the process². Hepatocytes will carry Sewage treatment; in fact, it is the best example for the best recycling system³. A lot of different kinds of allopathic molecules are available in the market all of them are suffering from some are the other toxic effect, so an urgent need of developing a herbal medicine that has got both liver-protecting and nutritional value is required; hence an attempt has been made to screen the hepatoprotective activity of leaf extracts of *Mansoa alliacea* which is also called *Bignonia alliacea* Lam. It is commonly called Garlic vein⁴, which belongs to the Bignoniaceae family. Many tribal's used various parts of these plants for treating a wide range of infections⁵, boils on skin and skin disease⁶, boiling of the leaves were used for

the treatment of infectious disease, ringworm, tape-worm⁷, post-partum hemorrhage, malaria, diabetes, pneumonia, and aching⁸.

MATERIALS AND METHODS:

Collection of specimens: The leaves of *Mansoa alliacea* were collected from the nearby area of Guntur district fields in February 2019 and were authenticated by Prof. D. Ramakant Raju, retired botanist, Andhra University.

Preparation of plant extracts:

Collected leaves of *Mansoa alliacea* has been made into small pieces and dried under shade pulverized to coarse structure and sieved through # 20 successively soxhlated using solvents like Hexane and ethanol for 72 hrs. After the extraction process; extracts were made solvent-free using rota evaporator and stored in a vacuum desiccator. The yield was found to be 12.25%, 23.7%w/w for *Mansoa alliacea* hexane extract (M.A.H.E.) and *Mansoa alliacea* ethanolic extract (M.A.E.E.), respectively. The concentrate extracts were stored in an air-tight container until further use. Oral suspensions of the extracts were prepared at a dose of 100mg/ml using 5% aqueous gum acacia.

EXPERIMENTAL WORK:

Toxicity determination using acute model:

Swiss albino mice of 20-25 gms were selected for the study and were screened into control and test groups; each contains six animals each. The control group received vehicle (5% of normal saline), and the test groups received graded doses of extracts. The animals were observed from 4 hours, then occasionally up to 48 hours for signs of any behavioural changes and mortality, and LD₅₀ values were calculated⁹.

Determination of hepatoprotective activity:

The experimental protocol was approved by the animal ethical committee of TRR

Educational society, Hyderabad, Which was registered with the CPCSEA bearing a registration number: 1447/PO/EdI/CPCSEA.

Selection of animals: 150-200g Wistar albino rats were selected for the study and fed with a balanced diet and tap water ad libitum. 40-70% humidity was maintained with 12 hours of light. Control group I received vehicle 5% aqueous gum acacia, II Group considered as the toxic group was given with CCl₄ only, standard drug silymarin was given for Group III at a dose 50mg/kg b.w and Group IV received *Mansoa alliacea* hexane extract (M.A.H.E.) was given at 500mg/kg b.w dose. Group V received *Mansoa alliacea* ethanolic extract (M.A.E.E.) at a dose of 500mg/kg b.w. All the animals received respective doses for a week period last day that is on 7th day carbon tetrachloride at a dose of 0.25mg/100g b.w was given to the groups of II to V after 30 minutes of the respective drug treatments. Blood samples were collected by puncturing retro-orbital plexus¹⁰. Serum parameters were tested. Animals were sacrificed and liver was collected for histopathological examination.

Assessment of liver function:

Assessment of liver function was done by studying changes in biochemical parameters. Viz Serum glutamic oxaloacetate transaminase (S.G.O.T.)/ (A.S.T.) and serum glutamic pyruvic transaminase (S.G.P.T.)/ (A.L.T.) were estimated by Reitman and Frankel method¹¹ Total bilirubin; Alkaline phosphatase were also estimated.

Statistical analysis¹²:

ANOVA and Dunnett's multiple comparisons were used for statistical analysis. Significance difference was checked using $P < 0.05$ were considered significant, and mean +/- S.E.M. were taken as significant. Percentage protection was calculated by considering the Percentage protection, exhibited by the plant extract and was checked by determining the difference between the toxic group and control group mean values.

Histopathological aspect: For the histopathological study, the liver from each animal was removed after dissection and preserved in 10% formalin. Five micron Sections of livers stained with the dye using eosin and hematoxylin for histopathological consideration.

Table:1 Results of *Mansoa alliacea* Lam phytochemical studies:

| S.no | Chemical constituents | M.A.H.E. | M.A.E.E. |
|------|----------------------------|----------|----------|
| 1 | Alkaloids | - | + |
| 2 | Amino acids | - | + |
| 3. | Saponins | + | - |
| 4 | Carbohydrates | + | - |
| 5 | Flavonoids | - | + |
| 6 | Mucilage | + | - |
| 7 | Proteins | - | + |
| 8 | Starch | + | - |
| 9 | Steroids and triterpenoids | - | + |
| 10 | Glycosides | - | + |
| 11 | Lignan's | - | - |

+ = presence of constituents, - =absence of constituents

M.A.H.E.: *Mansoa alliacea* Hexane extract shown presence of saponins, carbohydrates, mucilage, starch, as positive.

M.A.E.E.: *Mansoa alliacea* ethanolic extract shown positive for alkaloids, amino acids, flavonoids, steroids, triterpenoids and glycosides.

Table:2 Effect of *Mansoa alliacea* leaf extracts on serum biological parameters in CCL₄ induced liver damage model.

| Groups | SGOT(U/L) | SGPT(U/L) | ALP (U/L) | TB (mg/dl) |
|------------------------------|-------------------|------------------|------------------|--------------------|
| Control Group -I | 33.492 ±0.5820 | 27.750 ±0.5064 | 16.597 ± 0.4301 | 0.4433 ± 0.01022 |
| Toxic control Group-II | 95.465 ±0.462** | 78.412 ±0.6377** | 51.055 ±0.4419** | 2.222 ±0.1085** |
| Standard Group-III (25mg/kg) | 26.686 ± 0.4688** | 21.138 ±0.3145** | 15.381 ±0.2197** | 0.4717 ±0.004773** |
| M.A. H.E (500mg/kg) | 119.4 ±0.85* | 107.11 ±0.26* | 103.25 ±0.89* | 10.94 ±0.02* |
| M.A. E.E (500mg/kg) | 24.21 ±0.21** | 37.24 ±0.31** | 26.19 ±0.40** | 0.51 ±0.21** |

Values are mean±SEM, n=6

**P<0.001, when compared with the control group,

* P<0.01, when compared with Toxic Group.

Table-3 Percentage protection in biochemical parameters due to treatment with leaf extracts of *Mansoa alliacea*.

| Groups | Percentage protection of Biochemical parameters | | | |
|--------------------|---|--------|--------|-----------------|
| | SGOT | SGPT | ALP | Total Bilirubin |
| Standard Silymarin | 95.42% | 92.68% | 99.04% | 83.02% |
| M.A.H.E (500mg/kg) | 25.22% | 23.14% | 35.22% | 35.11% |

| | | | | |
|-----------------------|--------|--------|--------|--------|
| M.A.E.E (500mg/kg) | 94.32% | 92.81% | 98.22% | 82.71% |
|-----------------------|--------|--------|--------|--------|

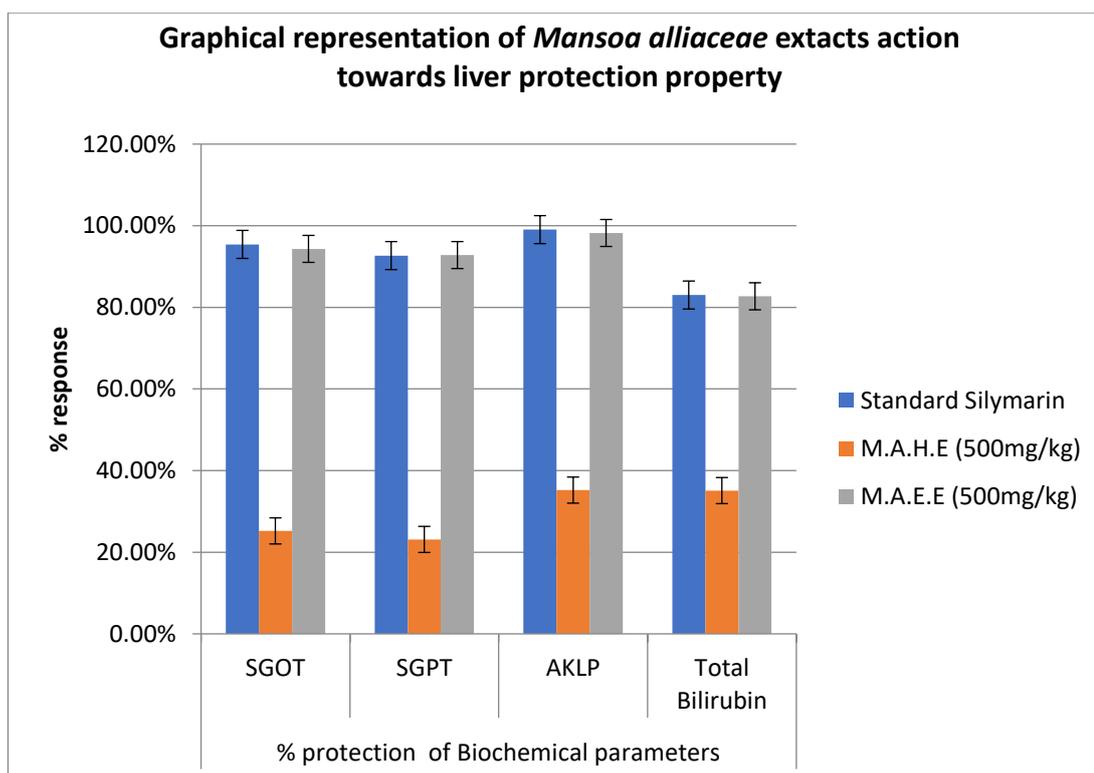
RESULTS AND DISCUSSION:

The hepatoprotective work of **Mansoa alliacea** was validated from this experimental study towards liver damage. From the results of acute toxicity studies, it was found that up to a dose of 5000mg/kg per oral was found to be safe. 1/10th dose was taken for efficacy determination towards liver failure assessment. Hepatic damage was assessed by inducing carbon tetrachloride as hepatotoxin. Useful for detection of early damage of hepatic cells.

The experimental results found that raised levels of S.G.O.T., S.G.P.T. and total bilirubin were detected in the toxic group. Pre-treatment with leaf extracts and silymarin in test groups and standard groups, respectively, daily for seven days showed a significant(p<0.01) protective effect against CCl₄ induced hepatotoxicity compared to the toxicant group. It is the best hepatoprotective action could be seen from the results, percentage protection in silymarin pre-treated group in the biochemical parameters, S.G.O.T., S.G.P.T., A.L.P., T.B. were found to be 95.42%, 92.68%, 99.04%, 83.02%, respectively, whereas in the **Mansoa alliacea** Ethanolic extract at a dose of 500mg/kg b.w has shown highly significant action as 94.32%, 92.81%, 98.22% and 82.71% respectively. Hexane extract-treated group has exhibited least significant action 25.22%, 23.14%, 35.22%, 35.11%.

Graph:1

Percentage inhibition of liver biochemical parameters of *Mansoa alliacea* extracts In CCl₄Induced Hepatotoxicity Model



Hence the ethanolic extract of *Mansoa alliacea* at the dose of 500mg/kg was showing significant hepatoprotective activity. The hepatoprotective activity of *Mansoa alliacea* could be due to Flavonoids¹³, proteins, and glycosides in case of ethanolic extract, which were reported to have hepatoprotective and antioxidant properties.

Histopathological sections of the liver in rats

Normal/control group

Fig:1 Histopathological section of the liver in Animals treated as Control Group-I shows normal cellular structure.

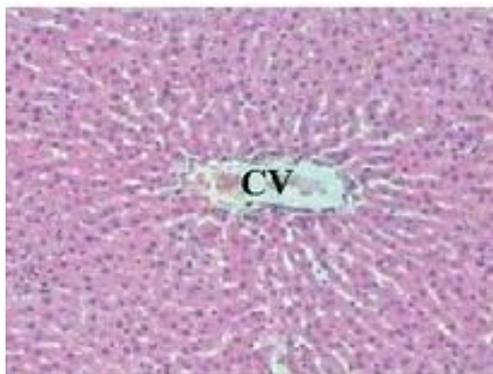


Fig:2: Histopathological section of the liver in Group II animals treated as Toxic group, treated with carbon tetrachloride showing central lobular vein with high amount of necrosis.

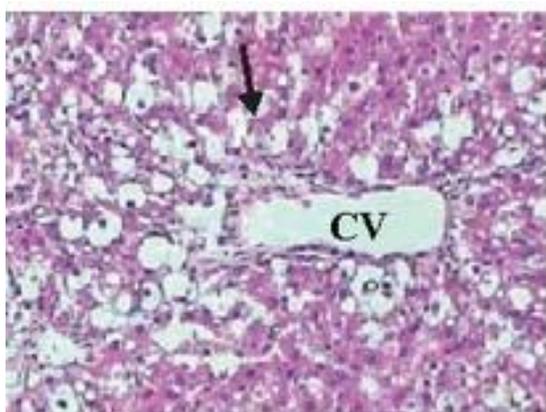


Fig :3 Histopathological section of the liver in Group III animals treated as a standard group, treated with carbon tetrachloride +Silymarin at a dose (50mg/kg b.w).

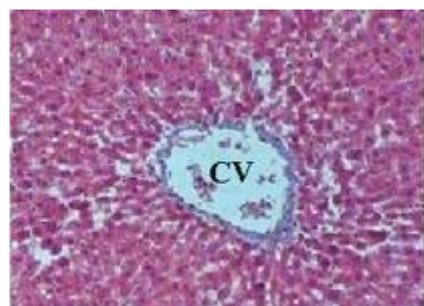


Fig:4 Histopathological section of the liver in Group IV animals treated with carbon tetrachloride + Hexane extract of *Mansoa alliacea* at a dose (500mg/kg b.w).

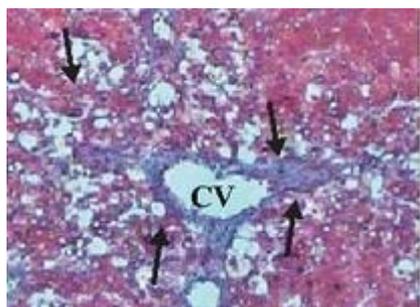
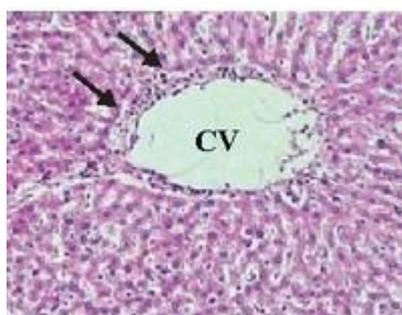


Fig:5 Histopathological liver sections in Group V animals treated with carbon tetrachloride + ethanolic extract of *Mansoa alliacea* at a dose (500mg/kg b.w).



Histopathological studies support the biochemical parameters in proving the hepatoprotection. Normal cell structure, prominent nucleus, well-defined cytoplasm in the control group. liver cell necrosis, fatty acid accumulation were identified in the toxic group liver histology. kupffer cell hyperplasia¹⁴, crowding of central vein and apoptosis. Hexane extract-treated group of *Mansoa alliacea* exhibited similar cellular structure like toxic group, loss of nucleus structure vacuolization, kupffer cell hyperplasia and apoptosis. Ethanolic extract-treated group of *Mansoa alliacea* exhibited similar to that of standard group regaining of cell structure well brought out central vein was identified. Out of two extracts, the ethanolic extract of *Mansoa alliacea* had shown very high significant potential Hepatic recovery at a dose of 500 mg/kg. b.w.

CONCLUSION: From this work, we can conclude that the folklore usage of *Mansoa alliacea* as a liver protective drug has been validated, it is useful in treating different liver infections and diseases.

ACKNOWLEDGEMENT

The authors wish to thank the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), for supporting this work. The authors wish to thank TRR College of Pharmacy, for providing facilities to conduct this work. The author would also like to thank colleagues for their support to complete research work.

CONFLICT OF INTEREST

The authors have no conflict of interests to disclose other than what has been

acknowledged above.

RESOURCES FOR FUNDING; NIL

REFERENCES:

1. Nwidi LL, Elmorsy E, Obama YI, Carter WG. Hepatoprotective and antioxidant activities of *Spondias mombin* leaf and stem extracts against carbon tetrachloride-induced hepatotoxicity. *Journal of Taibah University Medical Sciences* 2018;13(3):262-271.
2. Giovannini, P. Medicinal plants of the Achuar (Jivaro) of Amazonian Ecuador: Ethnobotanical survey and comparison with other Amazonian pharmacopoeias. *Journal of Ethnopharmacology*. 2015, 164 78–88.
3. T.Satyanarayana, B.Gangarao , Ch.K.V.L.S.N.Anjana Male, G.Surendra. Hepatoprotective Activity of Whole Plant Extract of *Vigna Mung* Linn Against Carbon Tetrachloride Induced Liver Damage Model *International Journal of Pharmacy and Biological Sciences*,2012; 2(3):256-63.
4. Aswini, D, Prabakar, K.; Rajendran, L.; Karthikeyan, G.; Raguchander, T. Efficacy of new EC formulation derived from garlic creeper (*Adenocalymma alliaceum* Miers.) against anthracnose and stem end rot diseases of mango. *World J Microbiol Biotechnol*. 2010, 26:1107–1116
5. Pagani, E.; Pagania, Santos, J.F.L.; Rodrigues, E. Culture-Bound Syndromes of a Brazilian Amazon Riverine population: Tentative correspondence between traditional and conventional medicine terms and possible ethnopharmacological implications. 2017, *Journal of Ethnopharmacology* 203 (2017) 80–89
6. Abhilash G, Maheswari YJ, Gopal A, Chanda D. Review on some medicinal plants with hepato-protective activities. *Res Rev J Pharmacogn Phytochem* 2014;22:33
7. Rane J, Jadhao R, Bakal RL. Liver diseases and herbal drugs: A review. *J Innov Pharm Biol Sci* 2016;3:24-36.
8. Zoghbi, M.dG.B.; Andrade, E.H.A.; Maia, J.G.S. Volatile constituents from *Adenocalymma alliaceum* Miers and *Petiveria alliacea* L., two medicinal herbs of the Amazon. 2009, *Flavour Fragr. J*. 2002; 17: 133–135. DOI: 10.1002/ffj.1051
9. Pires, F.B.; Dolwitsch, C.B.; Dal Prá, V.; Monego, D.L.; Schneider, V.M.; Loose, R.F.; Petra Schmidt, M.E.; Bressan, L.P.; Mazutti, M.A.; Barcellos da Rosa, M. An Overview about the chemical composition and Biological Activity of Medicinal species found in the Brazilian Amazon. *Journal of Applied Pharmaceutical Science*. 2016, Vol. 6 (12), pp. 233-238, December
10. Aayadi H, Mittal SPK, Deshpande A, Gore M, Ghaskadbi SS. Protective effect of geraniin against carbon tetrachloride induced acute hepatotoxicity in Swiss albino mice. *Biochemical and Biophysical Research Communications* 2017; 487: 62-67.
11. Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C, et al. A novel model of CCl₄-induced cirrhosis with ascites in the mouse. *J Hepatol* 2009;51:991–9.
12. Ahn M, Kim J, Bang H, Moon J, Kim GO, Shin T. Hepatoprotective effects of allyl isothiocyanate against carbon tetrachloride-induced hepatotoxicity in rat. *ChemicoBiological Interactions* 2016;254:102-108.
13. Iqbal SS, Mujahid M, Kashif SM, Khalid M, Badruddeen AM, Bagga P, Akhtar J, Rahman MA. Protection of hepatotoxicity using *Spondias pinnata* by prevention of ethanol-induced

oxidative stress, DNA-damage and altered biochemical markers in Wistar rats. Integr Med Res. 2016;5:267–75.

14. Ellman G. Tissue sulphhydryl. Arch Biochem Biophys 1959;82:70-77.