International Journal of Research in Medical Sciences
*Yengkhom NS et al. Int J Res Med Sci. 2017 Mar;5(3):806-810*
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20170501

**Original Research Article**

# Hepatoprotective effect of aqueous extract of *Melothria perpusilla* against carbon tetrachloride induced liver injury in albino rats

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**Received:** 21 January 2017
**Accepted:** 31 January 2017

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## ABSTRACT

**Background:** In absence of reliable hepatoprotective drug in modern medicine, the traditional herbal medicines have been emphasized. Present study was designed to assess hepatoprotective effect of aqueous extract of *Melothria perpusilla* (AEMP) against carbon tetrachloride ($CCl_4$) induced liver injury.
**Methods:** Five groups of animals with 6 rats in each were treated for 7 days. Group I received 1% gum acacia in distilled water (1 ml/200 g p.o.) daily. Group II, III, IV and V received $CCl_4$ in liquid paraffin (1 ml/kg s.c.) on day 2, 4 and 6. Group III, IV and V were treated respectively with silymarin (100 mg/kg p.o.), AEMP- 200 and 400 mg/kg p.o. daily. On day 8, liver injury was assessed by measuring serum ALT, AST, ALP and bilirubin.
**Results:** ALT, AST, ALP and bilirubin were significantly reduced in groups receiving both $CCl_4$ and AEMP when compared with $CCl_4$ treated group.
**Conclusions:** AEMP produced hepatoprotective effect against $CCl_4$ induced liver injury.

**Keywords:** Carbon tetrachloride, Hepatoprotective, Liver injury, *Melothria perpusilla*

## INTRODUCTION

Liver is prone to injury from an enormous array of therapeutic and environmental chemicals as it is the major site for metabolism and detoxification in the body. Injury may result from direct toxicity through conversion of the xenobiotics to active toxins, or by immune mechanisms.[1] A moderate liver cell injury may not be reflected by measurable change in its metabolic functions due to its regenerative capacity. Liver disorders depend on various factors such as nutritional, biochemical, bacteriological, viral and environmental aberrations. The impairment of its function is generally caused by xenobiotics, protozoal or viral infections, and excessive exposure to various pharmacological or chemical agents. Depending upon the severity of cellular injury, there can be development of acute hepatitis that may lead to chronic hepatitis and cirrhosis or malignant lesions in untreated cases.[2]

The true incidence of drug induced hepatotoxicity is difficult to estimate because of under reporting, difficulties in detection or diagnosis, and incomplete observation of persons exposed.[3] Drug-induced liver injury poses a major clinical problem and has become the leading cause of acute liver failure and transplantation in Western countries.[4] In fact, the condition turns out to be an issue not only to the health care professionals, but also to the pharmaceutical industry and drug regulatory agencies.[5] In most cases, there is no effective treatment other than stopping the offending agent and providing general supportive care. The drugs used in the treatment

of liver diseases are often inadequate and sometimes, the drugs may cause serious side effects. In absence of the reliable hepatoprotective drug in the modern medicine, there has been growing focus to evaluate scientific basis of the traditional herbal medicines which are claimed to be hepatoprotective.[6]

In Manipur, India the traditional medicine practitioners use specific plants for treatment of different ailments in the form of fresh preparation, decoction and powder of the whole or parts of the plant. *Melothria perpusilla* (Family- Cucurbitaceae) also known as "Lamthabi" in Manipuri is one of the plants used by the traditional practitioners as a cure for hepatic and renal ailments.[7] The plant is a monoecious, perennial climber with tendrils. Leaves are heart-shaped and lobed with distant spiny teeth on the margin. Flowers are small and yellow. Fruits look like miniature watermelons, and taste like cucumber. The vegetative parts of this plant are boiled with sugar candy in water and used for jaundice treatment.[8] However, despite such popular use among the traditional healers, there has been no substantial scientific data of the plant to support the claim. Therefore, we take up the study to evaluate hepatoprotective property of aqueous extract of *Melothria perpusilla* (AEMP) in rats using carbon tetrachloride ($CCl_4$) induced liver injury model.

## METHODS

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal after getting approval of the Institutional Animal Ethics Committee, RIMS, Imphal, India (No.1596/GO/a/12/CPSCEA).

### *Chemicals and drugs*

Carbon tetrachloride ($CCl_4$) was procured from SRL Pvt. Ltd., Mumbai, India. Assay kits for estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (total and direct) were purchased from Avecon Healthcare Pvt. Ltd., Parwanoo, India. Diethyl ether (Merck Specialities Pvt. Ltd., Mumbai) was used as anaesthetic agent. Silymarin (Batch No.SIBD0057, Micro Labs Ltd., Himachal Pradesh, India) was employed as the standard drug and gum acacia (Hi Media, Mumbai) as suspending agent.

### *Preparation of plant extract*

*Melothria perpusilla* (Blume) Cogn. (Family: Cucurbitaceae) was collected from the Bishnupur district of Manipur, India during the month of August, 2014. The plant was identified and authenticated by Dr. P.K. Singh, Professor, Department of Life Sciences, Manipur University. The shade dried aerial parts of the plant were crushed with mixer grinder and powdered material was extracted with distilled water using soxhlet apparatus. The brownish extract so obtained was put in an evaporating dish and evaporated to dryness on hot water bath. The dried extract was scraped out and preserved in an airtight glass container in the refrigerator for experimental use. The yield was approximately 14.1% w/w.

### *Phytochemical study*

The qualitative phytochemical tests[9] revealed presence of flavonoids, tannins and steroids in the aqueous extract of *Melothria perpusilla.*

### *Acute toxicity study*

Acute toxicity test was carried out as per the OECD guidelines 423 in female albino rats.[10] The rats were fasted for overnight with water *ad libitum*. Then, AEMP was administered to the fasted rats at a dose of 300 mg/kg p.o. Food was withheld for further 3-4 hours and observed once in every 30 min during the first 24 hours and thereafter, daily for a period of 14 days for any mortality. As there was no mortality, the procedure was repeated with higher dose of 2000 mg/kg and animals were observed for mortality and toxic symptoms. It was observed that the dose of 2000 mg/kg produced no mortality or toxic symptoms in the treated animals and considered safe. Two doses of 200 mg/kg (1/10th of the maximum test dose) and 400 mg/kg of AEMP were chosen for the study.

### *Experimental animals*

Healthy albino rats of either sex weighing 150-200g were obtained from the animal house, RIMS, Imphal, India. The rats were housed in polypropylene cages and acclimatized in the departmental animal room at room temperature with the natural light and dark cycle for 7 days prior to the experiment. The animals were fed on standard diet with free access to water.

### *Treatment protocol*

The method of Achliya GS et al was followed with minor modifications.[11] The overnight fasted rats were divided into 5 groups of 6 rats in each and treated for 7 days as follows: Group I (Normal control): 1% gum acacia in distilled water (1ml/200g p.o.), Group II (Toxic control): $CCl_4$ (1ml/kg s.c), Group III (Standard): Silymarin (100 mg/kg p.o.) + $CCl_4$ (1ml/kg s.c), Group IV (Test 1): AEMP (200mg/kg p.o.) + $CCl_4$ (1ml/kg s.c), Group V (Test 2): AEMP (400 mg/kg p.o.) + $CCl_4$ (1 ml/kg s.c).

The calculated doses of AEMP and silymarin were given as suspension in 1% gum acacia in distilled water (DW) at the dose of 1 ml/200 g p.o. daily for 7 days. Liver injury was induced in the group II, III, IV and V animals with $CCl_4$ in liquid paraffin (1:2) mixture (1ml/kg s.c.) on day 2, 4 and 6. Group I animals were also given liquid paraffin (1 ml/kg s.c.) on day 2, 4 and 6 while the group

II animals were administered 1% gum acacia in DW (1 ml/200 g p.o.) daily for 7 days.

***Blood sample preparation***

On the 8th day, blood samples were collected by orbital venous sinus puncture in sterile sample collection tubes under ether anaesthesia.[12] The collected samples were allowed to stand for 30 min at room temperature and then centrifuged at 2500 rpm for 5 min to obtain serum specimens.[13]

***Assessment of hepatic injury***

Liver injury was assessed by estimating serum levels ALT, AST, ALP and bilirubin (total and direct) using the test kits and colorimeter. The estimations were done as per the guidelines of the kit manuals.

***Analysis of results***

The values were expressed as mean ± SEM and analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test using SPSS version 16. The significance level was set at $p<0.05$.

## RESULTS

***Effect on serum ALT, AST and ALP levels***

There was significant increase ($p<0.001$) in hepatic enzymes- ALT, AST and ALP in the $CCl_4$ treated group (group II) when compared with the normal control (group I). In the silymarin (100 mg/kg) and AEMP (200 mg and 400 mg/kg) co-treatment groups i.e. group III, IV and V, the hepatic enzymes were significantly reduced ($p<0.001$) when compared with the $CCl_4$ alone treated group (group-II). However, the doses of 200 and 400 mg/kg of AEMP could not reduce the enzymes as low as in the silymarin treated group (group-III). AEMP at the dose of 400 mg/kg was more effective ($p<0.001$) to bring down AST and ALT levels when compared with 200 mg/kg dose (Table 1).

***Effect on serum bilirubin***

Both the total and direct bilirubin levels were significantly raised in the CCl4 alone treated group (group II) when compared with the normal control (group I). In the silymarin (100 mg/kg) and AEMP (200 mg and 400 mg/kg) co-treatment groups i.e. group III, IV and V, the total and direct bilirubin levels were significantly ($p<0.001$) reduced when compared with the $CCl_4$ treated group (group-II). Significant differences in the bilirubin levels were observed in the silymarin versus AEMP - 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.05$) treated groups. However, bilirubin levels observed between the groups receiving 200 and 400 mg/kg of AEMP did not differ significantly (Table 2).

**Table 1: Effect of aqueous extract of *Melothria perpusilla* on CCl4 induced AST, ALT and ALP levels.**

| Group | AST (IU/L) | ALT (IU/L) | ALP (IU/L) |
|---|---|---|---|
| I | 46.00±1.60 | 51.00±1.96 | 33.57±1.79 |
| II | 121.96±2.76* | 130.00±1.15* | 84.15±1.42* |
| III | 54.67±2.07† | 61.33±1.14† | 37.95±1.40† |
| IV | 87.33±1.79†‡ | 100.35±1.73†‡ | 51.45±1.94†‡ |
| V | 76.83±1.63†‡§ | 93.50±1.48†‡§ | 48.82±2.29†‡ |

Values are mean ± SEM, n=6 in each group. *p<0.001 when compared with normal control, †p<0.001 when compared with group II, ‡p<0.001 when compared with group III, §p<0.001 when compared with group IV. Group I: normal control; Group II: $CCL_4$ treated; Group III: silymarin (100 mg/kg) + $CCl_4$ treated; Group IV: AEMP (200 mg/kg) + $CCl_4$ treated; Group V: AEMP (400 mg/kg) + $CCl_4$ treated.

**Table 2: Effect of aqueous extract of Melothria perpusilla on CCl4 induced serum bilirubin levels.**

| Group | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) |
|---|---|---|
| I | 1.08±0.09 | 0.52±0.07 |
| II | 3.00±0.06* | 2.77±0.08* |
| III | 1.30±0.10† | 0.73±0.08† |
| IV | 2.20±0.09†‡ | 1.40±0.10†‡ |
| V | 1.90±0.08†§ | 1.23±0.11†§ |

Values are mean ± SEM, n=6 in each group; *p<0.001 when compared with normal control, †p<0.001 when compared with group II. ‡p<0.01 when compared with group III. §P<0.05 when compared with group III. Group I: normal control, Group II: $CCl_4$ treated, Group III: silymarin (100 mg/kg) + CCl4 treated, Group IV: AEMP (200 mg/kg) + CCl4 treated, Group V: AEMP (400 mg/kg) + CCl4 treated.

## DISCUSSION

$CCl_4$ induced liver injury is one of the classical models for studying hepatoprotective potential of drugs.[14] $CCl_4$ causes hepatic injury through generation of reactive radicals by the action of cytochrome P450. The reactive radicals destroy endoplasmic reticulum and activate mitogen activated protein kinase (MAPK) and sphingomyelinase. Later, the damage is through increased expression of various pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-1 (IL-1), cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB).[15,16,17]

When the liver cells are injured, its enzymes spill into the blood, causing elevated levels of ALT, AST, ALP, γ-glutamyl transpeptidase, sorbitol dehydrogenase, glutamate dehydrogenase and lactate dehydrogenase. Bilirubin, the endogenous product derived from the degradation of haemoglobin, builds up in the blood and extracellular fluid as its excretion is impaired. Usually, ALT and AST or in combination with total bilirubin are estimated for assessment of hepatocellular injury in rodents and non-rodents.[18]

Silymarin, a flavonolignan is a popular drug to treat various liver disorders. It helps in the maintenance of optimal redox status of the cell by activating a range of antioxidant enzymes and non-enzymatic antioxidants mainly via transcription factors, including Nrf2 (Nuclear factor-erythroid 2-related factor-2), NF-κB and activation of an array of vitagenes responsible for synthesis of protective molecules. NF-κB is a key transcriptional factor for numerous genes involved in the regulation of inflammation, immune system, cell differentiation, survival and apoptosis.[19]

The hepatoprotection provided by silymarin is attributed to its activity against lipid peroxidation as a result of free radical scavenging and ability to increase cellular reduced glutathione, ability to increase membrane stability, regulation of nuclear expression by means of a steroid-like effect and inhibition of transformation of stellate hepatocytes into myofibroblasts.[20]

Several studies revealed that $CCl_4$ treatment increased levels of AST, ALT, ALP and bilirubin.[21-26] Similarly, in present study there was significant rise of ALT, AST, ALP and bilirubin levels in the $CCl_4$ treated groups signifying the induced liver injury. The co-treatment of $CCl_4$ with silymarin, and also with AEMP reduced liver enzymes and bilirubin (Table 1 and 2). These observations strongly suggested that AEMP provided protection against $CCl_4$ induced liver injury. The hepatoprotective activity might be attributed to its phytochemicals such as flavonoids and steroids. The flavonoids are polyphenolic compounds and known antioxidants.[27,28] Moreover, various plant-derived polyphenols are also reported to suppress TNF-α and NF-κB associated inflammatory pathways both *in vitro* and *in vivo*.[19]

## CONCLUSION

Therefore, it can be concluded that aqueous extract of *Melothria perpusilla* produced hepatoprotective effect of against CCl4 induced liver injury. The protection afforded could be due to its antioxidant and anti-inflammatory properties. More in depth studies such as histopathological changes in liver, assessment of antioxidants and pro-inflammatory cytokines would be important to substantiate the hepatoprotective potential.

## ACKNOWLEDGEMENTS

Authors would like to respectfully acknowledge the contribution of Dr. PK Singh, Professor, Department of Life Sciences, Manipur University in the identification of the study plant.

*Funding: No funding sources*
*Conflict of interest: None declared*
*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Yengkhom NS, Gunindro N, Kholi SM, Moirangthem RS, Rajkumari BD. Hepatoprotective effect of aqueous extract of *Melothria perpusilla* against carbon tetrachloride induced liver injury in albino rats. Int J Res Med Sci 2017;5:806-10.