*Anupam et al. / International Journal of Pharmacy & Therapeutics*, 7(2), 2016, 49-XXX.

e- ISSN 0976-0342
Print ISSN 2229-7456

**International Journal of Pharmacy & Therapeutics**

Journal homepage: www.ijptjournal.com

IJPT

# HEPATOPROTECTIVE POTENTIAL OF *METHANOL EXTRACT OF SORGHUM VULGARE L.*AGAINST CARBON TETRA CHLORIDE-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

**Anupam Kanti Bag[1*], Deepak Kumar[2], Shaik. Karimulla[3]**

[1]Department of Pharmacology, Annamacharya College of Pharmacy, Rajampet, YSR Kadapah-516126, A.P, India.
[2]Department of Pharmacognosy, Gokula Krishna College of Pharmacy, Sullurpeta-524121, SPSR Nellore Dt, A.P, India.
[3]Department of Pharmacology, Dr. K. V. Subbareddy Institute of Pharmacy, Kurnool-518218, A.P, India.

**ABSTRACT**

The methanolic extract of leaves of *Sorghum vulgare (L.)* was studied for the hepatoprotective effect using Carbon tetrachloride induced liver damage in wistar albino rats. The MESV at doses of 200 and 400mg/kg, p.o and the standard drug Liv.52 (40mg/kg, p.o) were administered for 7 days in $CCl_4$ intoxicated rats. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea. The biochemical changes and histopathological studies were observed on 8$^{th}$ day. MESV at tested doses significantly decrease *(P*<0.001) the elevated levels of the hepatic enzymes, total bilirubin and urea in a dose dependent manner after 7days. The MESV afforded significant protection against $CCl_4$ induced hepatocellular injury.

**Key Words:-** Hepatotoxicity, Hepatic Enzymes, *Sorghum vulgare (L.)*, Hepatoprotective, MESV.

## INTRODUCTION

The plant *Sorghum vulgare* L., known as Millet or Guinea Corn. Sorghum is generally classified under two varieties, saccharine and non-saccharine. The saccharine sorghums are not used for producing sugar owing to the difficulty of crystallization. The plant *Sorghum vulgare* L., (cv. Cholam), a grass species is widely cultivated for its edible grains across northern part of Tamil Nadu. It can grow in prolonged drought hit and arid soils with more root-to-leaf area. It belongs to Poaceae family. On the basis of the traditional use of the plant for treating hepatic disorders, but no previous pharmacological (or) clinical study was carried out to test the hepatoprotective effect of this plant (Bulusu Sitaram & Chunekar KC, 2006). Since the hepatoprotective effect of *Sorghum vulgare* L. has been experimentally not confirmed. Keeping this fact in view, the present study was undertaken to investigate the hepatoprotective activity of *Sorghum vulgare (L.)* leaves against carbon tetrachloride- induced hepatic damage in albino rats.

**Corresponding Author**

**Anupam Kanti Bag**
Email: - Baganupam1986@gmail.com

## MATERIALS AND METHODS

### Plant collection

The Plant material of *Sorghum vulgare* L. used for investigation was collected from Tirunelveli District, in the Month of August 2014. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

### Preparation of extracts

The leaves of *Sorghum vulgare* L. was dried in

shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of methanolic extract of *Sorghum vulgare* L. was found to be 11.5 % w/w.

**Preliminary phytochemical screening**

The phytochemical examination of methanol extract of leaves of *Sorghum vulgare* L. was performed by the standard methods (Harbone JP, 1973)**.**

***Experimental Animals***

Wister albino rats weighing between 180-250gm each maintained in a 12 h light/dark cycle at a constant temperature 25 ºC with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

***Acute toxicity study***

Acute toxicity study of methanol extract of *Sorghum vulgare L.* was determined by acute toxic class method of OECD guidelines. In acute oral toxicity study mortality was not observed up to 2000mg/kg body weight (OECD, 2002).

***Carbon tetrachloride-induced hepatotoxicity in rats***

The liver protective effect was evaluated using the carbon tetrachloride ($CCl_4$) model described by Visweswaram *et al*. (1994). Wistar albino rats (180-250gm) were divided into six groups of six rats each and were subjected to the following treatments: Group-I served as normal control received distilled water (1 ml/kg, p.o) for 7days. Group II -V received 0.75 ml/kg $CCl_4$ administered orally as single dose. After 36 hours, Groups III-V received MESV with doses of 200 and 400mg/kg, p.o and the standard drug Liv.52 with dose of 40mg/kg, p.o, respectively once daily for 7days. The blood was collected by puncturing the retro-orbital sinus of three rats from each group on $8^{th}$ day after the treatment respectively. From the collected blood samples, serum was separated to assess various biochemical parameters.

***Biochemical estimation***

The separated serum was subjected to estimate SGOT and SGPT by Reitman and Frankel method, alkaline phosphatase (ALP) and acid phosphatase (ACP) by Kind and King method**,** bilirubin by Malloy and Evelyn method and urea by Bousquet method (Reitman S, Frankel S., 1957; Kind PRN, King EJ, 1954; Malloy HT, Evelyn KA., 1937; Bousquet BF *et al*., 1971). The rats were then sacrificed by bleeding and the liver was carefully dissected, cleaned of extraneous tissue, and part of the liver tissue was immediately processed for histopathological investigation.

**Statistical analysis**

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

**RESULTS**

**Preliminary phytochemical screening**

The results of preliminary phytochemical screening of the methanol extract of leaves of *Sorghum vulgare* L. revealed that presence of alkaloids, flavonoids, glycosides, tannins, saponins, terpeniods and absence of steroids.

**Acute toxicity study**

Acute toxicity study in which the animals treated with the MESV at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

***Effect of MESV on $CCl_4$ – induced hepatotoxicity***

The results of MESV on carbon tetrachloride-induced hepatotoxicity were represented in Table 1. The $CCl_4$ only treated animals exhibited a significant increase ($P$<0.001) the levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) and also total bilirubin and urea when compared to the normal control group on $8^{th}$ day, indicating hepatocellular damage

The MESV at tested doses (group III-IV) produced a significant reduction ($P$<0.001) in the $CCl_4$-

Induced elevated levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea when compared to the $CCl_4$ only treated animals (group-II) (Table 1). Overall, MESV at tested doses significantly reduced the levels of hepatic enzymes, total bilirubin and urea in a dose dependent manner. After 7 days, the hepatic enzymes levels were almost restored to the normal after treating with MESV at the dose of 400mg/kg, p.o.

A standard drug, Liv.52 at a dose of 40 mg/kg (group-V) administered orally produced a significant reduction ($p<0.001$) compared to $CCl_4$ only treated animals (group-II) on 8$^{th}$ day and these protective effects almost close to MESV 400mg/kg, p.o.

**Table 1. Effect of MESV on $CCl_4$-induced alteration of hepatic enzymes, serum bilirubin and urea in rat liver after7 days**

| Design of Treatment | Biochemical parameters | | | | | |
|---|---|---|---|---|---|---|
| | **SGOT**(U/ml) | **SGPT**(U/ml) | **ALP** (KA Units) | **ACP**(KA Units) | **Bilirubin**(mg/dl) | **Urea**(mg/dl) |
| Group-I: Normal control(DW-1 ml/kg; p.o) | 48.32 ± 1.26 | 58.21 ± 0.26 | 15.62 ± 0.22 | 5.22 ± 0.27 | 0.74 ± 0.02 | 37.21 ± 1.52 |
| Group-II: $CCl_4$ (0.75 ml/kg; p.o) | 157.40 ± 1.33$^{*c}$ | 117.44 ± 1.31$^{*c}$ | 45.42 ± 0.31$^{*c}$ | 5.42 ± 0.13$^{*c}$ | 2.52 ± 0.34$^{*c}$ | 91.47 ± 0.34$^{*c}$ |
| Group-III: MESV (200 mg/kg; p.o) | 64.23 ± 0.31* | 72.02 ± 0.32* | 20.03± 0.24* | 3.22 ± 0.34* | 1.14 ± 0.22* | 47.13 ± 1.12* |
| Group-IV: MESV (400 mg/kg; p.o) | 55.14 ± 1.14* | 62.42 ± 0.14* | 14.54 ± 0.01* | 3.25 ± 0.12* | 1.22 ± 0.01* | 38.60 ± 1.41* |
| Group-V: Liv.52 (40 mg/kg; p.o) | 55.14 ± 2.21* | 62.12 ± 1.33* | 17.34 ± 0.32* | 3.19 ± 0.02* | 0.92 ± 0.32* | 35.26 ± 1.22* |

Values are Mean ± SEM of 6 animals each in a group. $^{*c}$ $P<0.001$ , when compared group I *Vs* group-II,
**P*<0.001, when compared group II Vs group I, III, IV, V and VI
MESV= methanol extract of *Sorghum vulgare*, $CCl_4$ = Carbon tetrachloride. DW=distilled water.

## DISCUSSION AND CONCLUSION

The present studies were performed to assess the hepatoprotective activity of methanol extract of *Sorghum vulgare* leaves in rats against carbon tetrachloride. It is well documented that carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of drugs or medicinal plants' extracts, by in vivo and in vitro techniques (Venkidesh R *et al*., 2010; Satheesh Kumar P, Kishor Kumar V, 2012). Carbon tetrachloride ($CCl_4$) is a potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to $CCl_3$ by liver cytochrome P450-dependent monooxygenases (Recknagel RO *et al*., 1989).

In our study, the biochemical changes were observed after 7 days. Thereby, it was found that, the administration of MESV at doses of 200 and 400mg/kg, p.o for 7 days resulted in significantly decreases the $CCl_4$-induced elevated levels of the hepatic enzymes SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) in a dose dependent manner. These results indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts. Whereas, the MESV extracts at tested doses decreases the $CCl_4$-induced elevated level of hepatic enzymes in rats, and its subsequent return towards near normalcy after 7days. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with $CCl_4$(Recknagel RO *et al*., 1989).

Bilirubin is the conventional indicator of liver diseases. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice (Girish S *et al*., 2004). These biochemical restorations may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation (Drotman RB, Lawhorn GT, 1978). The marked elevation of bilirubin and urea level in the serum of group II $CCl_4$ intoxicated rats were significantly decreased in the groups III-IV. MESV treated animals after 7 days of treatment, bilirubin and urea level in the serum $CCl_4$ intoxicated rats subsequently return towards near normalcy in the groups III-IV MESV treated animals. These results further substantiate *Sorghum vulgare* as a potent hepatoprotective agent.

It has been reported that Liv.52 protects liver from the hepatotoxicity of carbon tetrachloride. An appreciable protective effect was observed even after 7 days treatment using marketed product (Liv.52). The extent of production by extracts appeared to depend on the duration of treatment. Overall, these results suggest that the MESV could protect the liver against damage induced by $CCl_4$ when comparable with Liv.52 (Elumalai A *et al*., 2012; Amzad Hossaina M *et al*., 2012; Arshed Iqbal Dar *et al*., 2012). Further research is needed to isolate and purify the active principle involved in hepatoprotection of this plant as well as to confirm the mechanisms responsible for hepatoprotective activity. The present finding provides scientific evidence to the ethnomedicinal use of *Sorghum vulgare* in treating hepatic disorders.

**REFERENCES**

Amzad Hossaina M, Raj Nagoorub M, Abdullah Bin Gansau J. New flavone from the leaves of local medicinal plant *Corydyline terminal is* Kunth. *International Journal of Biological & Pharmaceutical Research,* 3(2), 2012, 223-226.

Arshed Iqbal Dar, Saxena RC, Bansal SK, Bharti Matadeen, Rahul Saxena. Protective effect of *Citrullus colocynthis* L. against polluted water induced hepatotoxicity in albino rats. *International Journal of Biological & Pharmaceutical Research,* 3(2), 2012, 240-244.

Bousquet BF, Julien R, Bon R, Dreux C. *Ann Biol Clin,* 29, 1971, 415.

Bulusu Sitaram, Chunekar KC. Bhavaprakasa of Bhavamisra - Original Text Along with Commentary and Translated Including Nighantu Portion, *A chapter on medicinal trees*. Varanasi: Chaukhambha Orientalia Publication; 2006, p. 443.

Drotman RB, Lawhorn GT. *Drug Chem Toxicol,* 1, 1978, 163-171.

Elumalai A, Chinna Eswaraiah M, Sindhura S, Rajendra D, Manikanta KVC and Rajkumar CH. Acute toxicity studies and antipyretic activity of a Poly herbal formulation. *International Journal of Biological & Pharmaceutical Research,* 3(1), 2012, 130-132.

Girish S, Achliya Sudhir, Wadodkar G, Avinash Dorle K. *J Ethnopharmacol,* 90, 2004, 229-232.

Harbone JP. Phytochemical Methods, A Guide to modern technique of plant analysis, Chapmann and Hall, London, 1973, 1-271.

Kind PRN, King EJ. *J. Clin. Path,* 7, 1954, 132-136.

Malloy HT, Evelyn KA. *J Biol Chem,* 119, 1937, 481-485.

OECD, (2002) Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris June, 2000.

Recknagel RO, Glende EA, Jr Dolak JA, Waller RL. *Pharmacol Ther,* 43, 1989, 139–154.

Reitman S, Frankel S. *Am J Clin Path,* 28, 1957, 56-62.

Satheesh Kumar P, Kishor Kumar V. *In-Vitro* Antioxidant activity of methanolic extract of *Atalantia monophylla* Linn bark. *International Journal of Biological & Pharmaceutical Research,* 3(1), 2012, 122-125.

Venkidesh R, Subhash C Mandal, Dilipkumar Pal, Mohana Lakshmi S, Saravanakumar A. Hepatoprotective activity of *smilax chinensis l.* In carbon tetrachloride induced hepatotoxicity in Rats. *International Journal of Biological & Pharmaceutical Research.* 2010; 1(2): 72-75.

Visweswaram D, Rajeswara Rao P, Satyanarayana S. *Indian J Pharmacol,* 26, 1994, 301 – 303.