International Journal of Research in Medical Sciences
Dass E et al. Int J Res Med Sci. 2018 Mar;6(3):802-807
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20180403

Original Research Article

Hepatoprotective effect of DL-methionine on diclofenac-induced hepatotoxicity in albino rats: an experimental study

Ervilla Dass*, Bhagya Manoj Sattigeri

Department of Pharmacology, Smt. Bhikhiben Kanjibhai Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed to-be University, Vadodara, Gujarat, India

Received: 20 January 2018
Accepted: 29 January 2018

***Correspondence:**
Dr. Ervilla Dass,
E-mail: ervilladass@gmail.com

ABSTRACT

Background: Liver is the main detoxifying organ, which is affected by most of the drugs and xenobiotic agents that could result in liver damage. The present study was designed to evaluate the hepatoprotective effect of DL-Methionine against experimentally induced liver injury in albino rats.
Methods: Hepatotoxicity was induced by administering high doses of positive control drug Diclofenac sodium in albino rats, which was confirmed by estimating Liver Function Tests. Hepatoprotective effect was determined by administering DL- Methionine concomitantly with positive control drug. Albino rats were administered with DL-Methionine (700 mg/kg and 1400 mg/kg) respectively as a single oral dose, concomitantly with positive control drug Diclofenac sodium (96 mg/kg and 240 mg/kg) respectively. After 24-hours of post-treatment, serum levels of the liver enzymes were evaluated to demonstrate the hepatoprotective effect of DL-Methionine on drug-induced hepatotoxicity, and all the liver samples were examined for the histopathological study.
Results: Significant increase in serum transaminase enzymes were observed by the positive control drug Diclofenac sodium. There was significant reduction in the serum transaminases on concomitant administration of DL-Methionine with Diclofenac sodium. Liver injury induced by positive control drug; and its protection with DL-Methionine was revealed by histopathological study. The combination of Diclofenac sodium and DL-Methionine showed no significant histopathological difference when compared to the normal liver section.
Conclusions: The results reveal that, DL-Methionine significantly prevented the rise in transaminases levels produced by hepatotoxic doses of the positive control drug.

Keywords: Diclofenac, Drug-induced hepatotoxicity, Liver injury, NSAIDs, Serum markers

INTRODUCTION

Liver as a major organ involved in drug metabolism is susceptible to the injury when exposed to drugs, chemicals and xenobiotics, which is generally indicated by the elevated levels of serum enzymes in the liver.[1] Chemicals that cause liver injury are called hepatotoxins. Hepatotoxicity could be idiosyncratic or non-idiosyncratic.[2] However, the drug-induced liver toxicity has been a major concern with the hepatotoxic drugs such as antitubercular drugs (Isoniazid, Rifampicin, Pyrazinamide); Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as Ibuprofen, Diclofenac, Sulindac, Aspirin, Paracetamol which are commonly used as, anti-inflammatory and analgesics antipyretic preparations.[3]

The major concern with the group of NSAIDs is they belong to the class of non-prescription and commonly used Over-the-Counter (OTC) preparations.[4] The toxicity induced by these drugs have been the major concern since most of these are used for the long-term treatment, which has been a cause of withdrawal of the preparations

from the market or their termination during the clinical trials.[5]

Hepatocytes death is a characteristic presentation that occurs in case of liver injury, mainly due to the fibrosis and necrosis, which are generally prevented by N-Acetylcysteine, particularly in case of Acetaminophen toxicity.[6-9,3]

Similarly, the essential amino acid, Methionine has also found to be a beneficial hepatoprotective agent which is also been proposed for the treatment of certain disease condition.[3,10-12] Hence, the present study was taken up to demonstrate its hepatoprotective effect on Diclofenac-induced hepatotoxicity.

METHODS

Albino rats of either sex weighing between 100-400gm were used. Each animal was used only once. The animals were housed separately in poly-propylene rat-cages under controlled environmental conditions temperature 24°±2°C and 55%±5%, relative humidity, in a 12-hour light-/dark cycle throughout the experiment, which were kept fasting for 24hours, before administering the drug.

The drugs and chemicals used were Diclofenac sodium and DL-Methionine from Aatur Instru Chem, Vadodara. The chemicals included 10% Formalin, Xylene, Hemotoxylin and Eosin stains. To evaluate the levels of liver enzymes, serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Serum Alkaline Phosphatase, Serum bilirubin-Direct and Indirect Bilirubin, Total Bilirubin; Serum Gamma-Glutamyl Transpeptidase (GGTP) the diagnostic kit reagents (Erba Diagnostics, Manheim) was used.

Diclofenac sodium in the doses of 96mg/kg and 240mg/kg are used as positive control drug for their hepatotoxic effects and DL-Methionine 700mg/kg and 1400mg/kg are used as the test drugs to evaluate their hepatoprotective action on toxicity induced by Diclofenac sodium; by evaluating the liver enzymes and histopathological studies of liver.

The animals were grouped into seven groups (n=6). Group I (Control) was treated with Distilled Water 10ml/kg; while Group II, and III were treated with Diclofenac 96mg/kg and 240mg/kg respectively, which were considered as positive control group to demonstrate their hepatotoxic action.

Group IV was treated with DL-Methionine 700mg/kg and Diclofenac 96mg/kg concomitantly, Group V was treated with DL-Methionine 700mg/kg and Diclofenac 240mg/kg concomitantly; Group VI was treated with DL-Methionine 1400mg/kg and Diclofenac 96mg/kg concomitantly and Group VII was treated with DL-Methionine 1400mg/kg and Diclofenac 240mg/kg concomitantly.

The 24-hour fasted albino rats were administered with Diclofenac sodium and DL-Methionine as mentioned above. Later, after 24-hours of post-treatment, 3ml of blood sample was collected from retro-orbital plexus by capillary method technique, under light ether anaesthesia; that was centrifuged at 3000rpm for 10minutes to obtain the serum that was subjected to analyse the levels of liver enzymes. Liver from each animal was immediately dissected out and cleaned with normal saline and was preserved into the specimen collection jars that contained 10% formalin. The liver samples were quickly fixed in 10% formalin and embedded in paraffin. Sections of about 4-6µm were stained with haematoxylin for 5minutes at room temperature; 15minutes later was counterstained with eosin for 2minutes; washed with xylene and blocked by eosin for histopathological studies and were observed under photomicroscope.

Statistical analysis

All the observed data were subjected for statistical analysis and the results were expressed as Mean± SEM. All calculations were performed using statistical software SPSS version 21.0 computer-based. Values were considered to be significant when P values were less than or equal to 0.05 ($P \leq 0.05$).

RESULTS

The positive control drug Diclofenac sodium in the dose of 96mg/kg and 240mg/kg showed significant rise (P value < 0.0001) of serum SGPT and SGOT level when compared to the control group.

On concomitant administration of DL-Methionine 700mg/kg with Diclofenac sodium 96mg/kg and 240mg/kg (Group IV and Group V) respectively; there occurred significant reduction ($p<0.05$) in the serum SGOT and SGPT levels as compared to control and positive control group. Similarly, a significant reduction in the serum SGPT as shown in Table 1 and Figure 1, and serum SGOT levels, as shown in Figure 2, was observed in Groups VI and VII, which were treated concomitantly with DL-Methionine 1400mg/kg and Diclofenac sodium 96mg/kg and 240mg/kg respectively.

However, in both the doses of DL-Methionine (700mg/kg and 1400mg/kg), with Diclofenac sodium 96mg/kg and 240mg/kg, there occurred no statistically significant changes in the other liver enzymes such as, Total Serum Bilirubin, serum Alkaline Phosphatase and serum Gamma-Glutamyl Transpeptidase (GGTP) levels as indicated in Table 1 and Figure 3, 4 and 5.

Histopathological examination

Gross appearance of liver

The gross appearance of liver of albino rats in control group showed reddish to brown colour. On administration

of positive control drug Diclofenac sodium, which were pale yellow; on concomitant administration of DL-Methionine with hepatotoxic drug Diclofenac sodium, showed near normal gross appearance of liver, of mild reddish-brown color.

Table 1: Hepatoprotective action of DL- methionine, on concomitant administration of positive control drug diclofenac sodium.

Group (n = 6)	Biochemical parameters of LFTS Mean± SEM values				
	SGPT (IU/L)	SGOT (IU/L)	Total serum bilirubin (µmol\L)	ALP (IU/L)	GGTP (IU/L)
Control DW 10ml/kg	32.83±2.91	126.00±15.07	0.70±0.08	106.17±23.15	2.33±0.56
Diclofenac 96 mg/kg	147.67±13.72***	1220.83±130.50***	1.07±0.12	153.83±32.01	3.03±1.40
Diclofenac 240mg/kg	236.50±24.01**	1490.00±168.88***	1.25±0.11	229.00±32.06	1.60±0.28
Diclofenac sodium 96mg/kg + DL-methionine 700mg/kg	57.17±5.19**	295.00±22.87**	0.95±0.08	151.17±8.42	2.42±0.30
Diclofenac sodium 240mg/kg + DL-methionine 700mg/kg	69.17±3.57**	395.83±20.95**	1.01±0.09	136.83±27.79	7.38±1.62
Diclofenac sodium 96mg/kg + DL-methionine 1400mg/kg	43.00±4.25***	225.17±9.27**	0.88±0.09	133.83±16.07	2.76±0.88
Diclofenac sodium 240mg/ml + DL-methionine 1400mg/kg	69.50±6.76**	301.83±22.76**	1.11±0.20	134.50±31.48	2.98±0.44

** p value < 0.05 = significant, **p <0.001 = highly significant and ***p value < 0.0001 = very highly significant, values are presented as Mean± SEM, Serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Total serum bilirubin, Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGTP) or γ-Glutamyl Transferase (GGT), DW = Distilled Water*

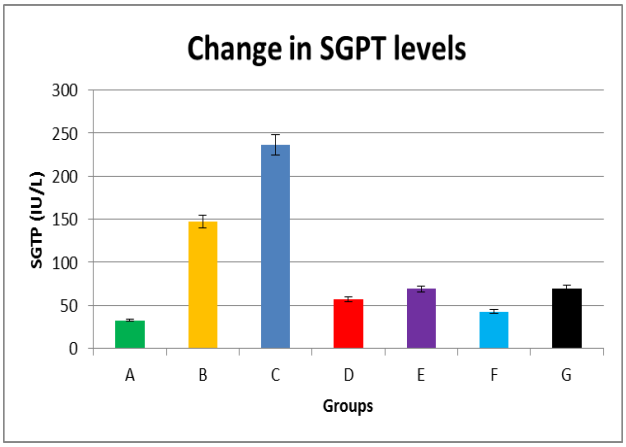


Figure 1: Changes in the Serum Glutamic-Pyruvic Transaminase (SGPT) levels on concomitant administration of DL-methionine and the positive control drug.

Microscopic examination of liver

The histopathological changes observed in the liver tissue in control group revealed normal liver architecture (Figure 6).

Diclofenac treated rats, shows mainly hepatocellular changes in the portal area, mainly microvesicular vacoulation, that is diffuse hepatic vacoulation degeneration, cytoplasmic vacoulation and sinusoidal dilatation.

Portal congestion was markedly seen in the hepatotoxic drug Diclofenac sodium, compared to those concomitantly administered with DL-Methionine. (Figure 7(a), 7(b) and 7(c)).

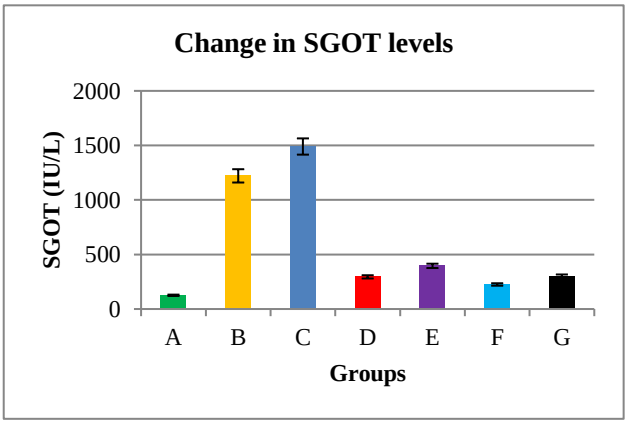


Figure 2: Changes in the Serum Glutamic-Oxaloacetic Aminotransferases (SGOT) levels on concomitant administration of DL-Methionine and the positive control drug.

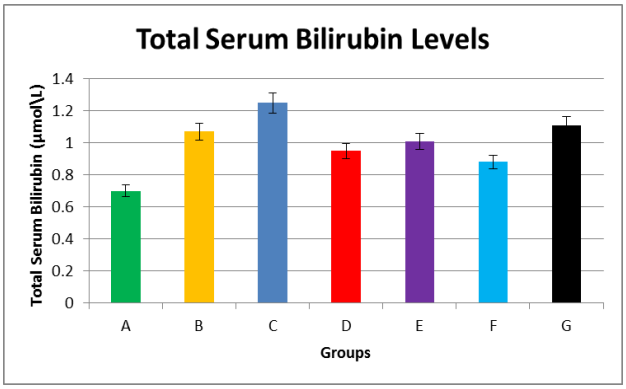


Figure 3: Changes in the Total Serum Bilirubin levels on concomitant administration of DL-methionine and the positive control drug.

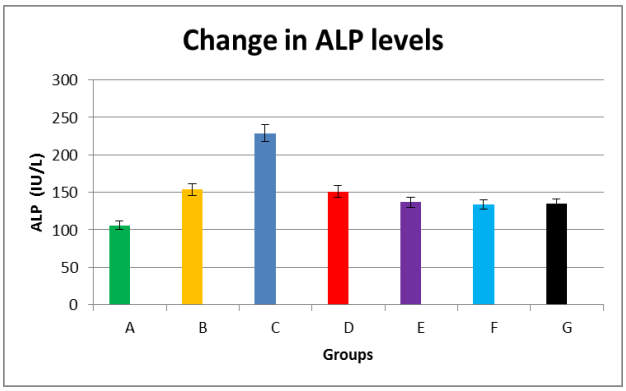
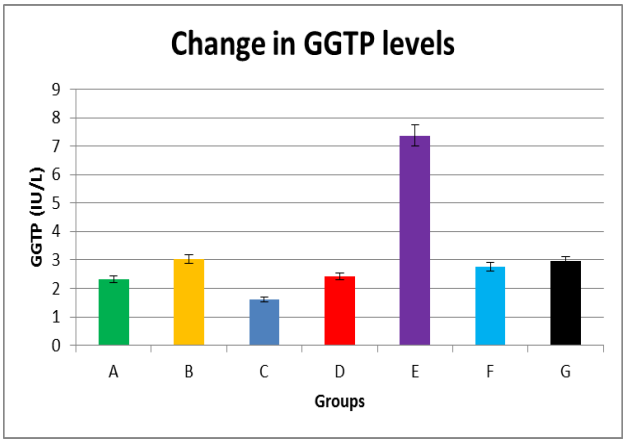


Figure 4: Changes in the serum Alkaline Phosphatase (ALP) levels on concomitant administration of DL-methionine and the positive control drug.



A = Control, B= Diclofenac 96 mg/kg, C= Diclofenac 240 mg/kg, D = Diclofenac sodium 96 mg/kg, + DL-Methionine 700 mg/kg, E = Diclofenac sodium 96 mg/kg + DL Methionine 1400 mg/kg, F = Diclofenac sodium 240 mg/ml + DL Methionine 1400 mg/kg (shown in Figure 1-5).

Figure 5: Changes in the Serum Gamma Glutamyl Transpeptidase (GGTP) levels on concomitant administration of DL-methionine and the positive control drug.

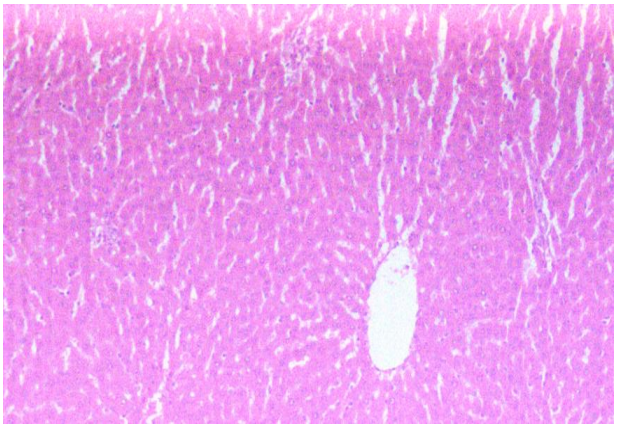


Figure 6: Liver sections from control rats showing central vein.

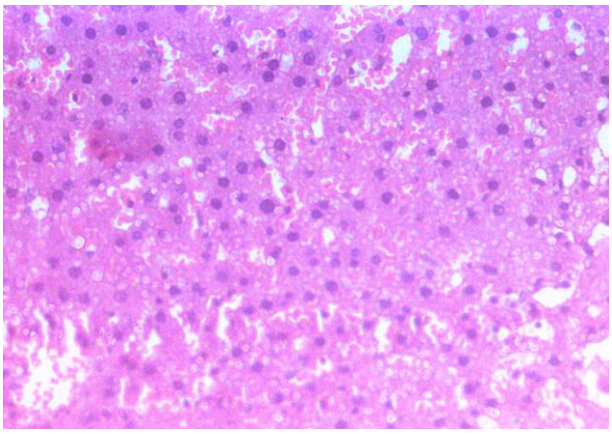


Figure 7(a): Liver section from diclofenac sodium 72mg/kg.

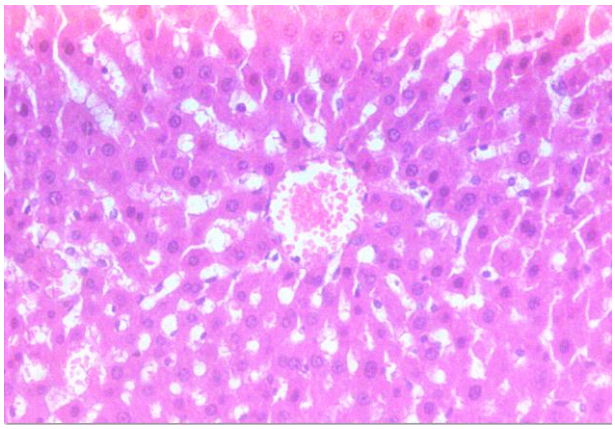


Figure 7(b): Liver section from diclofenac sodium 96mg/kg.

Diffuse hepatic vacoular degeneration was observed prominently in Diclofenac treated rats, which was reduced extensively in the DL-Methionine treated rats (Figure 8(a), 8(b), 8(c) and 8(d)).

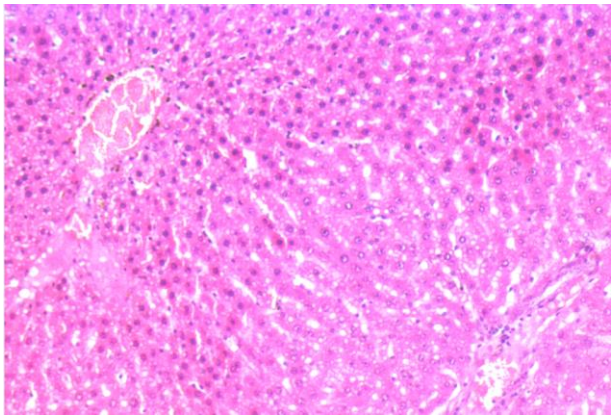


Figure 7(c): Liver section from diclofenac sodium 240mg/kg.

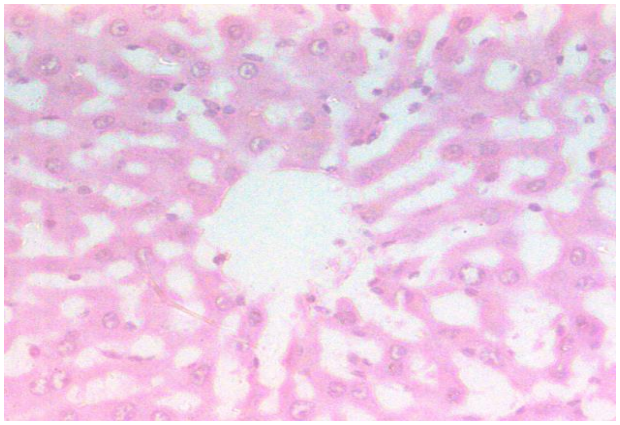


Figure 8(a): Liver section from diclofenac sodium 96mg/kg + DL-methionine 700mg/kg treated rats.

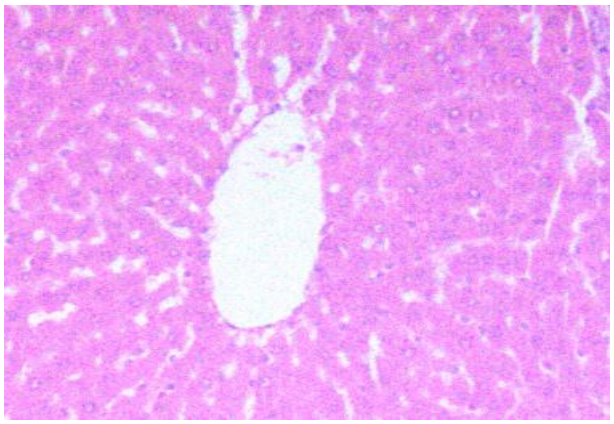


Figure 8(b): Liver section from diclofenac sodium 240mg/kg + DL-methionine 700mg/kg treated rats.

DISCUSSION

It was observed by the authors that, the positive control drug Diclofenac sodium 96mg/kg and 240mg/kg caused hepatotoxicity, which was indicated with the significant rise ($P < 0.05$) of serum enzymes both SGOT and SGPT levels. While on the concomitant administration of the positive control drug with DL-Methionine, it was observed that there occurred a significant reduction ($P < 0.05$) in serum levels of SGOT, SGPT in both the groups which received 96mg/kg and 240mg/kg of the hepatotoxic drug Diclofenac sodium.

This observation concurs with that of observations made by Dass E et al.[3] It also proves DL-Methionine to be a good hepatoprotective agent as shown by Anstt QM et al.[13]

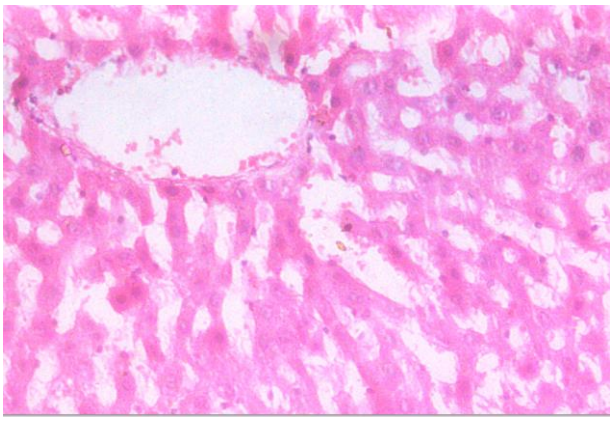


Figure 8(c): Liver section from diclofenac sodium 96mg/kg + DL-methionine 1400mg/kg treated rats.

CONCLUSION

With the observations made in the present study, we conclude that DL-Methionine is a hepatoprotective agent as it has protected the hepatotoxicity induced by Diclofenac sodium, a known NSAID to cause hepatotoxicity. Although, N-Acetylcysteine is an established hepatoprotective agent for paracetamol-induced hepatotoxicity, from the present study, it is evident that Methionine also has been a hepatoprotective agent on Diclofenac sodium which belongs to the class of NSAIDs.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Swapan Goswami, Professor, Department of Pathology for his extensive and valuable contribution and guidance pertaining to the histopathological examinations in the present study.

Authors also grateful to Mr. Devendra Waghasia, Statistician, Department of Community Medicine for his valuable guidance pertaining to the statistical analysis in the present study. Author are also grateful to Dr. Kirankumar, Tutor, Department of Pharmacology, for his valuable support.

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

REFERENCES

1. Maity T, Ahmad A. Protective effect of Mikania scandens (L.) Willd. against Isoniazid induced hepatotoxicity in rats. Int J Pharm Pharm Sci. 2012;4:466-9.
2. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis. 2009;29(4):337-47.
3. Dass EE, Shah KK. Paracetamol and conventional antimalarial drugs induced hepatotoxicity and its protection by methionine in rats. Indian J Exp Biol. 2000;(38):1138-42.
4. Parikh D, Sattigeri BM, Kumar A, Brahmbhatt S. A survey study on use of over the counter (OTC) drugs among medical students, nursing and clerical staff of a tertiary care teaching rural hospital. Inter J Res Med Sci. 2017;1(2):83-6.
5. Watkins PB. Drug safety sciences and the bottleneck in drug development. Clin Pharmacol Ther. 2011;89(6):788-90.
6. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest. 1998;102(3):538-49.
7. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther. 1973;187(1):211-7.
8. Vale JA, Meredith TJ, Goulding R. Treatment of acetaminophen poisoning. The use of oral methionine. Arch Intern Med. 1981;141 (3 Spec No):394-6.
9. Piperno E, Berssenbruegge DA. Reversal of experimental paracetamol toxicosis with N-acetylcysteine. Lancet. 1976;2(7988):738-9.
10. Leiber CS. S-Adenosyl-L-methionine: its role in the treatment of liver disorders. The American Journal of Clinical Nutrition. 2002;76(5):1183S-7S.
11. Mato JM, Lu SC. Role of S-adenosyl-L-methionine in liver health and injury. Hepatology. 2007;45(5):1306-12.
12. Purohit V, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, Kaplowitz N, et al. Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. Am J Clin Nutr. 2007;86(1):14-24.
13. Anstee QM, Day CP. S-adenosylmethionine (SAMe) therapy in liver disease: A review of current evidence and clinical utility. J Hepatology. 2012;57(5):1097-1109.

Cite this article as: Dass E, Sattigeri BM. Hepatoprotective effect of DL-methionine on diclofenac-induced hepatotoxicity in albino rats: an experimental study. Int J Res Med Sci 2018;6:802-7.