INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

HEPATOPROTECTIVE ACTIVITY OF *ARTOCARPUS HETEROPHYLLUS* LAM. LEAVES AGAINST THIOACETAMIDE INDUCED HEPATOTOXICITY ON WISTAR ALBINO RATS

Om Prakash [1]*, Ritika Srivastava [2], Rajesh Kumar [2], Rajesh Kumar [3]
[1]Goel Institute of Pharmacy & Sciences, Faizabad Road, Near Indira Canal, Lucknow, Uttar Pradesh, India
[2]Faculty of Pharmacy, Kamla Nehru Institute of Management and Technology, Sultanpur, Uttar Pradesh, India
[3]Faculty of Pharmacy, Ashoka Institute of Technology & Management, Paharia, Sarnath, Varanasi, Uttar Pradesh, India
*Corresponding Author Email: opverma2007@gmail.com

Article Received on: 11/01/16 Revised on: 24/01/16 Approved for publication: 08/03/16

DOI: 10.7897/2230-8407.07434

ABSTRACT

In the present study, the hepatoprotective activity of *Artocarpus heterophyllus* Lam. leaves was investigated against thioacetamide induced hepatotoxicity model in Wistar albino rats. The acute toxicity study showed that there is no mortality or adverse reaction at the fixed dose of 2000 mg/kg. The methanolic and aqueous extract at 250 and 500 mg/kg body weight respectively were administered daily to study the hepatoprotective effect in thioacetamide induced hepatotoxic model for the period of 10 days. It was observed that there was a remarkable decrease in bilirubin (Total and Direct), ALP, SGOT and SGPT level in treatment group as compared to the hepatotoxic group. In histopathological study, hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis was found in hepatotoxicity induced group whereas minimal inflammation with moderate portal triaditis and normal lobular architecture observed in extract treated groups. From the observation, it can be concluded that the methanolic as well as aqueous extract of *Artocarpus heterophyllus* Lam. have the potential to revert the hepatic injury induced by thioacetamide. Comparatively, the aqueous extract at 500 mg/kg b.w. resulted significant hepatoprotective effect as compared to methanolic extract at the same dose level. The standard drug silymarin was used for comparison.

Keywords: Hepatoprotective activity, *Artocarpus heterophyllus* Lam., Silymarin, Thioacetamide

INTRODUCTION

Liver diseases are serious ailments, which are classified as chronic hepatitis (inflammatory liver disease), hepatosis (non inflammatory disease) and cirrhosis i.e. degenerative disorder resulting in fibrosis of the livers[1]. Liver cirrhosis has become a serious health problem because of the wider use of prescribed medications with adverse reactions in modern life of today or the drug misuse. The current research has targeted on finding new therapeutic alternatives and analyzing their mechanism to get rid of the signaling routes and reduce the loss induced on the liver[2]. The numbers of compounds of natural origin are generally used as possible health care options and they are being experimented on various animal models[3, 4]. A large number of medicinal plants with hepatoprotective activity have been reported by several researchers[5, 6]. In absence of reliable hepatoprotective drugs in allopathic medical practices, herbs play a vital role in the management of various liver disorders. In continuation of search of hepatoprotective agents, Jackfruit was under taken to investigate about the active constituents and its medicinal value. *Artocarpus heterophyllus* Lam. (Jackfruit) is a species of tree of the mulberry family (Moraceae) is traditionally used to treat the liver cirrhosis[7]. It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, South-eastern Asia, Florida, Brazil, Australia and many Pacific Islands. It is a large, evergreen tree, 10-15 m in height, indigenous to the evergreen forests at an altitude of 450-1,200 m and cultivated throughout India. The plant is reported to possess antibacterial[8], anti inflammatory[9], anti-diabetic[10], antioxidant[11], anti-fungal[12] and immunomodulatory properties[13]. It is also used for the treatment of fever, boils, wounds, skin diseases, convulsions, diuretic, constipation, ophthalmic disorders and snake bite etc [14,15]. *Artocarpus heterophyllus* Lam. is reported to have various medicinal properties such as insecticidal, CNS stimulant, anti-cancer and treatment of chronic kidney diseases. It contains the various active constituents such as morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, cycloheterophyllin, artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophylol[16-18]. The plant also contains free sugar (sucrose), fatty acids, ellagic acid and some essential Amino acids like Arginine, Cystine, Histidine, Leucine, Lysine, Metheonine, Theonine, Tryptophan etc[19]. Even through the plant was traditionally used to treat liver disease, but there is no such documented evidence in relation to hepatoprotection. In view of this, it is of considerable interest to investigate the above mentioned plant with a hope to obtain a safe and potent hepatoprotective agent.

MATERIALS AND METHODS

Collection and identification of plant sample

The fresh leaves of *Artocarpus heterophyllus* Lam. leaves were collected from local area of Lucknow, India and was authenticated from Taxonomy Division of National Botanical Research Institute, Lucknow, India (Ref No: NBRI/CIF/74/2009).

Preparation of Plant Extract

The fresh leaves were washed with tap water, followed by distilled water and then air dried under shade at room temperature. The size of the leaves was reduced, powdered and about 50 g of powder was continuously extracted with methanol and water by soxhlet extractor. The residue was filtered and concentrated in rotary evaporator under reduced pressure at 40°C. The obtained dark brown to green color crude extract was stored in airtight container at 4°C for further studies[20, 21].

Chemicals

Thioacetamide was purchased from S.D Fine Chemicals Pvt. Ltd., India. Bilirubin, ALP, SGOT and SGPT kits were purchased from Ranbaxy diagnostics, New Delhi, India. All other chemicals used were of analytical grade unless otherwise stated.

Animals

Male albino rats, weighing between 150-200 gm were used in the study. Rats were housed in the departmental animal house at an ambient temperature of 25°C, under a 12 hour dark -12 hour light cycle for the whole period of the study. The animal experiments were carried out according to the guidelines of the animal ethics committee of the institute. All the experiments were carried out as per CPCSEA guidelines (Committee for the Purpose of Control and Supervision of Experiments on Animals) after obtaining the approval (Ethical committee No BBDNITM/IAEC/Clear/03/2009) from Institutional animal ethical committee.

Acute toxicity study

The acute toxicity of methanolic and aqueous extracts was determined using male Wistar rats (120-150 g). Acute toxicity was calculated as per OECD guidelines No. 420 and the LD_{50} of the test compounds was found at 2000 mg/kg b.w.

Thioacetamide-induced hepatotoxicity

Seven groups consisting of six animals each were used for the study. Group I was kept as control and received vehicle (5% Tween 80) only. Group II was kept as negative control received Thioacetamide (200 mg/kg) for eight days. Group III was kept as positive control and received standard drug silymarin (50 mg/kg) for eight days. Group IV and V received *Artocarpus heterophyllus* Lam. methanolic extract at an oral dose of 250 and 500 mg/kg respectively for eight days. Group VI and VII received *Artocarpus heterophyllus* Lam. aqueous extract at an oral dose of 250 mg/kg and 500 mg/kg respectively for eight days. All the groups except group I received a single oral dose of thioacetamide 200 mg/kg on the seventh day.

Biochemical and histopathological studies

After 48 h thioacetamide intoxication, the animals were sacrificed by mild anesthesia. Blood samples were collected in glass test tubes and allowed to coagulate for 20 min. Serum was separated by centrifugation at 3000 g for 20 min and used for evaluating biochemical parameters and liver tissue was sliced for histopathological studies. Biochemical parameters like Serum glutamate oxaloacetate transferase (SGOT), Serum glutamate pyruvate transferase (SGPT), alkaline phosphatase (ALP) and Serum bilirubin were estimated by reported methods[22, 25].

Statistical analysis

Results of the study were expressed as mean±SEM. The student *t*-test and analysis of variance (ANOVA) was used followed by Newman-Keul's multiple comparison tests to analyze the experimental data for its significance.

RESULTS

Histopathological studies

Control (Group I): Hepatocytes of the normal control group showed a normal lobular architecture of the liver (Figure. a).

Toxic Control Groups (II): Hepatocytic necrosis and inflammation region was observed in liver treated with thioacetamide (Figure. b).

Silymarin 50 mg/kg (Groups III): Silymarin pretreated group showed normal hepatocytes normal architecture (Figure. c).

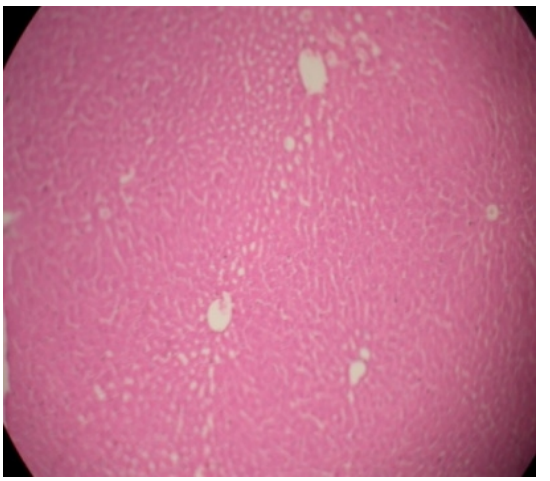
Methanolic extract 250 mg/kg and 500 mg/kg (Groups IV & V): Methanolic extract 250 mg/kg pretreated group showed minimal inflammation and moderate portal triaditis with their normal lobular architecture (Figure. d & e).

Aqueous extract 250 mg/kg and 500 mg/kg (Groups VI & VII): Aqueous Groups 250 mg/kg pretreated group showed remarkable reduction of inflammation with moderate portal triaditis and their lobular architecture was normal (Figure. f & g).

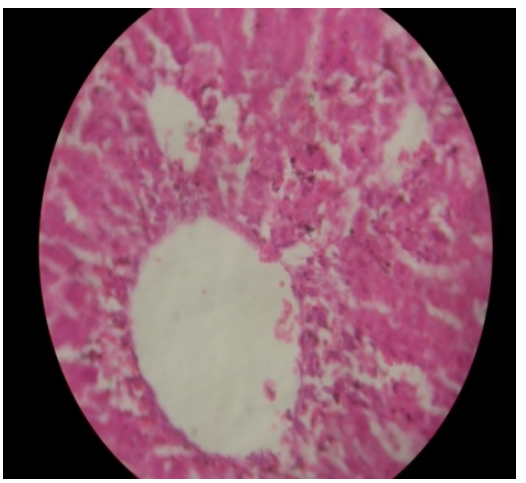
The phytochemical analysis of extract of leaves of *Artocarpus heterophyllus* Lam. confirmed the presence of various phytoconstituents *i.e.* alkaloids, flavonoids, glycosides, terpenoids and steroids. The extract is devoid of toxicity up to 2000 mg/kg in Wistar albino rats. In thioacetamide treated rats, the levels of Bilirubin (Total & Direct), SGOT, SGPT and Alkaline phosphatase were significantly elevated. Silymarin (50 mg/kg) showed a significant decrease in Bilirubin (Total & Direct), SGOT, SGPT and Alkaline phosphate level. Treatment with methanolic extract of *Artocarpus heterophyllus* Lam. at 250 mg/kg and 500 mg/kg showed a significant decrease in Bilirubin (Total & Direct), SGOT, SGPT and Alkaline phosphatase level. Similarly the treatment of aqueous extract at 250 and 500 mg/kg but 250 mg/kg showed a significant decrease in the above mentioned biomarkers. However, 500 mg/kg b.w had showed profound reduction of the above markers. The detailed result of the biochemical analysis is given in table 1. The histopathological studies of the normal liver sections showed the hepatic cells with well preserved cytoplasm, prominent nucleus and central vein (Figure. a). In rats treated with thioacetamide, the normal architecture of liver was completely lost with the appearance of centrilobular necrosis, lymphocytes infiltration of the periportal area and fatty changes were observed (Figure. b). The rats administrated with silymarin (50 mg/kg) exhibited the significant reversal of the hepatic damage caused by thioacetamide (Figure. c). Methanolic extract at dose of 250 mg/kg and 500 mg/kg had shown some improvement in damaged hepatocytes whereas the aqueous extract at 250 and 500 mg/kg had shown remarkable improvement in damaged hepatocyte which is evidenced from reversal of damaged hepatocytes to normal hepatic architectural pattern with mild hepatitis (Figure. d & e).

Table 1: Effect of leaves of *Artocarpus heterophyllus* Lam. on serum bilirubin (Total and Direct), ALP, SGOT and SGPT in thioacetamide treated rats

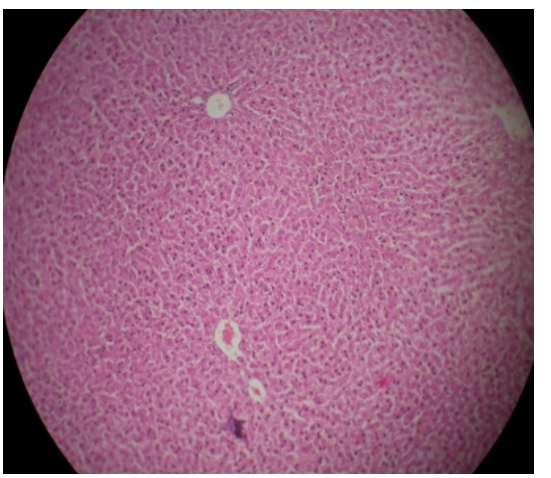
Treatment (mg/kg, *p.o.*)	Serum Bilirubin Total (mg/dl)	Serum Bilirubin Direct (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)
Group I	0.82±0.025	0.14±0.003	11.50±0.22	28±0.57	205±1.06
Group II	1.39±0.050	0.39±0.007	53.83±1.19	87.17±2.92	316.8±4.82
Group III	0.88±0.011***	0.22±0.005***	19.00±0.73***	27.33±0.66***	195.7±1.14***
Group IV	1.25±0.025	0.37±0.005	51.50±0.22	85.0±0.36	310.7±1.11
Group V	1.18±0.024***	0.36±0.002***	50.33±0.66***	80.67±0.49***	300.3±0.80***
Group VI	1.20±0.027**	0.34±0.003**	44.67±1.25**	74.33±0.88**	290.5±1.47**
Group VII	1.00±0.007***	0.31±0.007***	41.83±1.19***	70.17±0.74***	279.7±4.52***
Values are in Mean ± SEM. Number of animals in each group (N=6). ***p< 0.001 Vs Group I. **p<0.01 Vs Group II. *p<0.05 Vs Group II					



A



B



C

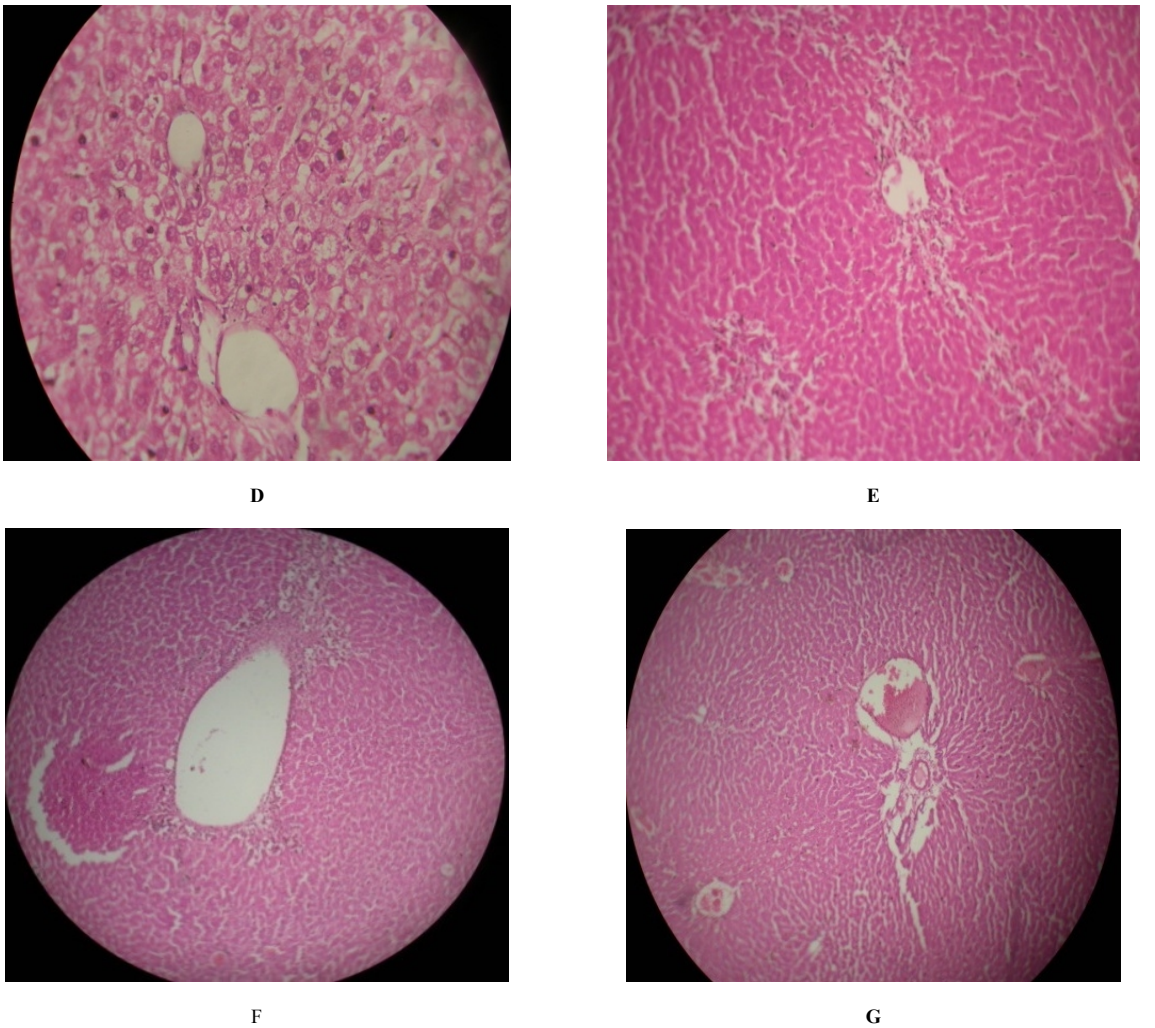


Figure 1: **Histopathology of methanol and aqueous extracts of leaves of *Artocarpus heterophyllus* Lam.**
(A): A normal lobular architecture of Hepatocytes of showed the normal control lobular architecture group showed liver.
(B): Hepatocytes of the thioacetamide treated the liver cell necrosis and inflammation also observed in the centrilobular region with portal triaditis.
(C): Hepatocytes of the Silymarin pretreated group showed normal hepatocytes and their lobular architecture was normal.
(D): Hepatocytes of the methanol 250mg pretreated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal.
(E): Hepatocytes of the methanol 500mg pretreated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal.
(F): Hepatocytes of the aqueous extract 250 mg pretreated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal.
(G): Hepatocytes of the aqueous extract 500mg pretreated group showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal.

DISCUSSION

The present investigation reports the hepatoprotective effects of methanolic and aqueous extracts of leaves of this plant. Hepatotoxicity model in Wistar rats was produced by administering thioacetamide (200 mg/kg on 8th day) orally. Thioacetamide is biotransformed by CYP2E1 enzymes located in the microsomes of liver cells and convert it to a highly reactive toxic intermediate known as thioacetamide sulphur dioxide through oxidation[26], inducing hepatotoxicity in experimental animals and different grades of liver damage including nodular cirrhosis, production of pseudo lobules, proliferation of hepatic cells, and necrosis of parenchyma cells[27]. Thioacetamide induces hepatic damage via its metabolite, $TASO_2$, which damages the macromolecules of hepatocytes causing damage of DNA molecules, oxidation of protein molecules, and peroxidation of the cell membrane biomolecules[28, 29]. Administration of thioacetamide at doses of 100-300 mg/kg, *i.p.* results in the hepatic damage in animals[30, 31]. The serum SGOT, SGPT, Bilirubin and ALP are reliable markers to assess the extent of hepatic damage. The study was carried out to find out the effect of methanolic as well as aqueous extract at 250 & 500 mg/kg in thioacetamide pretreated rats. The results revealed that both methanolic & aqueous extract have profound hepatoprotective effect at two different dose levels. The control of hepatic injury is more prominent in aqueous extract as compared to methanolic extract. It can be correlated that more flavanoids may be present in aqueous extract rather than methanolic extract[32]. The protective effect is more significant in 500 mg/kg rather than 250 mg/kg of aqueous

as well as methanolic extract. This interpretation was ascertained based on the reduction in level of biochemical markers as well as reduction of hepatic damage from histopathological studies. The experimental results were statistically treated for its significance at $p<0.001$, $p<0.01$ and $p<0.05$ level.

CONCLUSION

The detailed study of the plant proved that it has a significant hepatoprotective effect on liver injury induced by thioacetamide. In mechanistic point of view, it can be proposed that the hepatoprotective effect may be resulted due to inhibition of peroxidation of bio membranes. This study provides the evidence that the above mentioned plant could be used for the development of phytomedicine against liver ailments. However, a detailed study is needed to reach a concrete conclusion to unveil the phytoconstituents which is responsible for the hepatoprotective activity.

ACKNOWLEDGEMENT

Authors are thankful to Shri Vinod Singh, Manager, Kamla Nehru Institute of Management & Technology, Sultanpur, Uttar Pradesh, India for providing the laboratory facilities to carry out the research work. Authors are also thankful to the Director, Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology & Management, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow for the support extended to conduct animal studies.

REFERENCES

1. Sreejith G, Jayasree M, Latha PG, Suja SR, Shyamal S, Shine VJ, et al. Hepatoprotective activity of *Oxalis corniculata* L. ethanolic extract against paracetamol induced hepatotoxicity in Wistar rats and it's *in vitro* antioxidant effects. *Indian J Exp Biol*. 2014; 52(2): 147-152.
2. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Peer I, Floratos A, et al. HLA-B5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nature Genetics 2009; 41(7): 816-819.
3. Yang H, Sung SH, & Kim YC. Two new hepatoprotective stilbene glycosides from Acer mono leaves. Journal of Natural Products 2005; 68(1): 101-103.
4. Wang N, Li P, Wang Y, Peng W, Wu Z, Tan S, et al. Hepatoprotective effect of *Hypericum japonicum* extract and its fractions. Journal of Ethnopharmacology 2008; 116: 1-6.
5. Alshawsh MA, Abdulla MA, Ismail S, Amin ZA. Hepatoprotective effects of *Orthosiphon stamineus* extract on thioacetamide-induced liver cirrhosis in rats. Evidence based Complementary and Alternative Medicine 2011; 2011:103039.
6. Amin ZA, Bilgen M, Alshawsh MA, Ali HM, Hadi AHA, Abdulla MA. Protective role of *Phyllanthus niruri* extract against thioacetamide-induced liver cirrhosis in rat model. Evidence Based Complementary and Alternative Medicine 2012; 2012:241583.
7. Gupta AK & Tandon N. *Review on Indian Medicinal Plants.* Vol. 3 (Indian Council of Medical Research, New Delhi, India) 2004, 182-200.
8. Khan MR, Omoloso AD & Kihara M. Antibacterial activity of *Artocarpus heterophyllus*, Fitotherapia 2003; 74: 501-505.
9. Fang S C, Hsu C L & Yen G C. Anti-inflammatory effects of phenolic compounds isolated from the fruits of *Artocarpus heterophyllus*. Journal of Agricultural and Food Chemistry 2008; 56: 4463-4468.
10. Fernando MR, Wickramasinghe N, Thabrew MI, Ariyananda PL & Karunanayake EH. Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. Journal of Ethnopharmacology 1991; 31: 277-282.
11. Ko FN, Cheng ZJ, Lin CN & Teng CM. Scavenger and antioxidant properties of prenyl flavones isolated from *Artocarpus heterophyllus*. Free Radical Biology & Medicine 1998; 25: 160-168.
12. Trindade MB, Lopes JL, Costa AS, Moreira AC, Moreira RA, Oliva MLV et al. Structural characterization of novel chitin-binding lectins from the genus *Artocarpus* and their antifungal activity. Biochim Biophys Acta 2006; 1764: 146-152.
13. Kabir S. Jacalin: a Jackfruit (*Artocarpus heterophyllus*) seed derived lectin of versatile applications in Immunobiological research. Journal of Immunological Method 1998; 212(2): 193-211.
14. Anonymous. The Wealth of India, *A dictionary of Indian raw materials and industrial products*. (Publication and information directorate CSIR, New Delhi, India); 1985: 445-453.
15. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. (National Institute of Science Communication & Information Resources, New Delhi, India); 2002: 27.
16. Chun NL, Chai ML. Heterophylol, a phenolic compound with novel skeleton from *Artocarpus heterophyllus*. Tetrahedron letters 1993; 34: 8249-8250.
17. Chung MI, Lu CM, Huang PL, Lin CN. Prenylflavonoids of *Artocarpus heterophyllus*, Phytochemistry 1995; 40: 1279-1282.
18. Rama RAV, Varadan M, Venkataraman. Colouring matter of the *A. hetetophyllus*. Indian J Chem. 1973; 11: 298-299.
19. Pavanasasivam G, Uvais M, Sultanbawa S. Cycloartenyl acetate, cycloartenol and cycloartenone in the bark of *Artocarpus species.* Phytochemistry 1973; 12: 2725-2726.
20. Parameswari SA, Chetty CM, Chandrasekhar KB. Hepatoprotective activity of *Ficus religiosa* leaves against isoniazid+rifampicin and paracetamol induced hepatotoxicity. Pharmacognosy Res. 2013; 5(4): 271-276.
21. Akanni OO, Owumi SE, Adaramoye OA. In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from *Artocarpus altilis*, *Ficus exasperate* and *Kigelia Africana*. Asian Pac J Trop Biomed. 2014; (Suppl 1): S492–S499.
22. Asha VV, Sheeba MS, Suresh V, Wills PJ. Hepatoprotection of *Phyllanthus maderaspatensis* against experimentally induced liver injury in rats. Fitoterapia 2007; 78: 134-135.
23. Vogel GH. Drug Discovery and Evaluation and Pharmacological Assays. 2nd ed. New York: Springer Verlag Berlin Heidelberg; 2002: 936-939.
24. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 56(28):56-63.
25. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem. 1937; 119: 481-490.
26. Kim KH, Bae JH, Cha SW, Han SS, Park KH, Jeong TC. Role of metabolic activation by cytochrome P450 in thioacetamide-induced suppression of antibody response in male BALB/c mice. Toxicology Letters 2000; 114: 225-235.
27. Sadasivan S, Latha PG, Sasikumar JM, Rajashekaran S, Shyamal S, Shine VJ. Hepatoprotective studies on *Hedyotis*

corymbosa (L.) Lam. Journal of Ethnopharmacology 2006; 106: 245-249.
28. Chilakapati J, Korrapati MC, Hill RA, Warbritton A, Latendresse JR, Mehendale HM. Toxicokinetics and toxicity of thioacetamide sulfoxide: a metabolite of thioacetamide. Toxicology 2007; 230: 105-116.
29. Fabeqat I. Dysregulation of apoptosis in hepatocellular carcinoma cells. World J Gastroenterol. 2009; 15: 513-520.
30. Shyamal S, Latha PG, Suja SR, Shine VJ, Anuja GI, Sini S, et al. Hepatoprotective effect of three herbal extracts on aflatoxin B1- intoxicated rat Liver. Singapore Med J. 2010; 51: 326-331.
31. Shapiro H, Ashkenazi M, Weizman N, Shahmurov M, Aeed H, Bruck R. Curcumin ameliorates acute thioacetamide-induced hepatotoxicity. Journal Gastroenterol Hepatol. 2006; 21: 358-366.
32. Di CG, Mascolo N, Izzo AA, Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sciences 1999; 65: 337-353.

Cite this article as:

Om Prakash, Ritika Srivastava, Rajesh Kumar, Rajesh Kumar. Hepatoprotective activity of *Artocarpus heterophyllus* Lam. leaves against thioacetamide induced hepatotoxicity on wistar albino rats. Int. Res. J. Pharm. 2016;7(4):24-29 http://dx.doi.org/10.7897/2230-8407.07434

Source of support: Nil, Conflict of interest: None Declared