INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

ATTENUATING EFFECT OF *UVARIA NARUM* (DUNAL) WALL. LEAVES ON THIOACETAMIDE INDUCED HEPATIC INJURY

Patel Jagir R*, Moses Samuel Rajan, Ashok Shenoy, Prima D'souza, K. Gunjan

Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Mangalore, Karnataka, India

*Corresponding Author Email: jagsp070@gmail.com

Article Received on: 17/07/13 Revised on: 21/07/13 Approved for publication: 01/08/13

DOI: 10.7897/2230-8407.04842

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT

The objective of the present study was to evaluate the hepatoprotective potential of *Uvaria narum* leaf extract at two different doses (200 and 400 mg / kg b.w.) against thioacetamide induced Hepatic injury. Thioacetamide at dose of 100 mg / kg induced liver toxicity, which was assessed by quantifying the serum hepatic biomarkers like SGPT, SGOT, ALP, total and direct bilirubin, serum biochemical parameters like albumin, total protein, cholesterol, triglycerides, glucose and urea and oxidative parameters like glutathione, catalase, superoxide dismutase and lipid peroxidation in hepatic tissue. Further study was supported by histopathology studies. The results revealed that Silymarin (100 mg / kg b.w.) and *Uvaria narum* leaf extract at both doses prevented the elevation of serum hepatic biomarkers and also altered serum biochemical parameters dose dependently. Whereas decrease in lipid peroxidation and elevation in catalase, glutathione and superoxide dismutase was observed in Silymarin and extract treated animals. Histopathological changes in hepatic tissue also supported the dose dependent protective effect of *Uvaria narum* leaf extract. The results were comparable to reference standard Silymarin.

Keywords: Hepatoprotective, Biochemical parameters, Thioacetamide, Histopathology.

INTRODUCTION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any type of injury (due to systemic drugs, food preservatives, agrochemicals, and addiction of alcohol) or impairments of its functions may lead to many complications in one's health.[1] Hence liver diseases are among the most serious health aliments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorders resulting in fibrosis of liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, Alfatoxin, carbon tetrachloride, paracetamol, chlorinated hydrocarbons, thioacetamide etc), excess consumption of alcohol, infections and auto immune disorder. So it has become very much necessary to protect the liver from all these agents[2]. In the absence of reliable hepatotoxic drugs in modern medicine, herbs and plants play an important role in the management of several diseases. In Ayurveda the ancient system of Indian medicine identifies liver disease quite early and recommend no of herbal remedies. Herbal medicine is gaining popularity since cheap, easily available and have rare or no side effect.[3] *Uvaria narum* (Dunal) wall. (Annonaceae family) is a medicinal plant widely distributed in foot hills of Western Ghats and is popularly used in ethnomedicine for the treatment of eczema and pityriasis. The plant showed the presence of phenols, tannins, antioxidants. The presence of these phytoconstituents in the plant has been attributed to its various medicinal properties. Literature survey reveals the plant may also be considered against ageing and other disease caused by free radicals, due to antioxidant property of plant.[4] Thioacetamide was originally used as fungicide to protect the decay of organs. It is recognized as a potent hepatotoxin and carcinogen in rats[5]. Therefore, in the present study this model of hepatitis in rats was used to assess the hepatoprotective effect of *Uvaria narum* leaves extract (UNLE).

MATERIALS AND METHODS

Plant material

The fresh leaves of *Uvaria narum* used for the present studies were collected from local areas of Mangalore, Karnataka, India. It was authenticated by Mr. Dinesh Nayak Advisor (Green belt), Mangalore SEZ ltd. A voucher specimen has been maintained in our lab for further reference. The leaves were shade dried, pulverized into coarse powder and were stored in air tight containers. Extraction of leaves was done by soxhlation process using ethanol as a solvent, until colourless solvent appeared in siphon tube. Further extract was dried and kept in desiccator for further study.

Animals

Healthy Wistar albino rats (150–200 g) of either sex were used for the experiment. They were maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60 ± 5 % and 12 h light / dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. (Ref no: SCP/CPCSEA/P12/F150/2011).

Drugs and Chemicals

All chemicals and solvents used in the study were of analytical grade. Thioacetamide (Loba chime, Mumbai, India) and other chemicals like Nitroblue tetrazolium, Phenazine methasulphate, NADH, Thiobarbituric (Himedia suppliers) and all estimation kits were obtained from Agapee distributers.

Acute Toxicity Studies

The acute oral toxicity study of was performed as per the OECD guideline No. 425. Limit test was performed at dose 2000 mg / kg. Animals were observed after dosing individually at least once during the 30 minutes for 4 h, periodically during the first 48 h and daily thereafter for 14 days for signs of toxicity and mortality, if any.[6]

Thioacetamide (TAA) Induced Hepatic Injury

Experimental Design

Wistar rats of either sex weighing between 150-200 g were divided into five groups of six animals each. For the first nine days of study Group I and II was treated with vehicle 1 ml / kg / day and were fed with normal feed and water. Group III animals were treated with Silymarin (100 mg / kg) and group IV and V were treated with *Uvaria narum* leaf extract (200 mg / kg and 400 mg / kg extract was suspended in 1 % w/v gum tragacanth) respectively for 9 days. All the treatment was done post orally. On 9th day, all the animals except Group I were intoxicated by the administration of TAA 100 mg / kg s.c. After 48 h of intoxication by TAA administration, blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Animals were sacrificed and liver was dissected out and used for histopathological studies.[7]

Assessment of Hepatoprotective Activity

Biochemical parameters and tissue antioxidants

The collected blood was used for estimation of serum biochemical parameters like SGOT, SGPT, ALP, total (BILT) and direct bilirubin (BILD), total protein (TOT PRO), albumin (ALB), cholesterol (CHO), triglycerides (TG), urea and glucose contents were estimated by using commercially available reagents kits (AGAPPE) according to manufactures instruction. Liver tissue was estimated for lipid peroxidation (LPO)[8], reduced glutathione[9] (GSH), catalase[10] (CAT) and superoxide dismutase[11] (SOD) were assayed according the methods described by previous workers and were expressed in absorbance and % increase or decrease.

Relative organ weight analysis

On the 9th day the animals were sacrificed, liver, spleen, left lung, heart, kidney were removed, washed with ice cold saline and were immediately weighed and liver volume was also measured, all the weights of organs were expressed as g / 100 g B.W.[12]

Table 1: Effect of UNLE on Serum Hepatic Biomarkers in Thioacetamide Induced Hepatic Injury

Groups	Treatment	SGPT (U / l)	SGOT (U / l)	ALP (U / l)	BILT (mg / dl)	(BILD mg / dl)
Normal control	Vehicle 1 ml / kg	71.32 ± 5.17	127.7 ± 4.70	384.7 ± 27.03	0.51 ± 0.04	0.12 ± 0.03
Toxic control	TAA 100 mg / kg	405.4 ± 50.95$^{###}$	223.5 ± 31.34$^{###}$	1015.7 ± 21.09$^{###}$	1.48 ± 0.21$^{###}$	0.42 ± 0.035$^{###}$
Standard	Silymarin 100 mg / kg	198.5 ± 15.77***	132.8 ± 6.69 **	484.3 ± 34.89***	0.51 ± 0.01***	0.11 ± 0.01***
Low dose	UNLE 200 mg / kg	259.3 ± 22.95**	161.4 ± 4.99*	656.1 ± 90.48**	0.60 ± 0.02 ***	0.18 ± 0.02***
High dose	UNLE 400 mg / kg	205.4 ± 12.26***	147.7 ± 2.70 **	534.8 ± 97.88***	0.55 ± 0.02 ***	0.15 ± 0.04***

All the values are Mean ± SEM, n = 6 One way ANOVA followed by Tukey's multiple comparison test. ns- $p > 0.05$, $^{###}p < 0.001$ compared to normal control, *$p < 0.05$,**$p < 0.01$, ***$p < 0.001$ compared to toxic control.

Table 2: Effect of Silymarin and UNLE on Serum Biochemical Parameters in Thioacetamide Induced Hepatic Injury

Groups	Treatment	TOT protein (g / dl)	ALB (g / dl)	TG (mg / dl)	CHO (mg / dl)	Urea (mg / dl)	Glucose (mg / dl)
Normal Control	Vehicle 1 ml / kg	6.93 ± 0.64	3.91 ± 0.22	95.3 ± 4.06	94.5 ± 5.70	43.33 ± 2.01	125.8 ± 15.72
Toxic control	TAA 100 mg / kg	3.93 ± 0.53$^{###}$	2.40 ± 0.14$^{###}$	190.8 ± 1.43$^{###}$	196.5 ± 31.5$^{##}$	61.50 ± 2.64$^{###}$	61.67 ± 3.76$^{###}$
Standard	Silymarin 100 mg / kg	7.71 ± 0.14***	3.53 ± 0.17***	133.7 ± 4.80***	93.13 ± 2.33**	37.83 ± 1.88***	110.2 ± 6.95**
Low dose	UNLE 200 mg / kg	6.68 ± 0.38***	3.26 ± 0.11*	166.3 ± 6.73**	104.3 ± 5.41**	39.33 ± 2.10***	94.50 ± 6.43
High dose	UNLE 400 mg / kg	7.13 ± 0.17***	3.33 ± 0.22**	143.4 ± 4.16***	97.33 ± 6.46**	36.00 ± 1.73***	100.3 ± 7.76*

All the values are Mean ± SEM, n = 6 One way ANOVA followed by Tukey's multiple comparison test. ns- $p > 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$ compared to normal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to toxic control

Table 3: Effect of Silymarin and UNLE on Relative Organ Weight (g / 100 g B.W.) in Thioacetamide Induced Hepatic Injury

Groups	Treatment	Liver weight	Liver Vol	Spleen weight	Kidney weight	Left lung weight	Heart weight
Normal Control	Vehicle 1 ml / kg	4.8 ± 0.26	4.0 ± 0.43	0.32 ± 0.03	0.64 ± 0.03	0.23 ± 0.01	0.37 ± 0.01
Toxic control	TAA 100 mg / kg	6.81 ± 0.32$^{###}$	6.2 ± 0.36$^{##}$	1.1 ± 0.21$^{###}$	1.37 ± 0.12$^{###}$	0.49 ± 0.01$^{###}$	0.66 ± 0.02$^{###}$
Standard	Silymarin 100 mg / kg	4.4 ± 0.33***	4.1 ± 0.36**	0.38 ± 0.01***	0.60 ± 0.04***	0.27 ± 0.01***	0.33 ± 0.02***
Low dose	UNLE 200 mg / kg	4.9 ± 0.13***	4.6 ± 0.28*	0.50 ± 0.07**	0.74 ± 0.05**	0.36 ± 0.04**	0.38 ± 0.01***
High dose	UNLE 400 mg / kg	4.8 ± 0.15***	4.4 ± 0.20**	0.46 ± 0.04**	0.71 ± 0.05***	0.30 ± 0.01***	0.35 ± 0.02***

All the values are Mean ± SEM, n = 6 One way ANOVA followed by Tukey's multiple comparison test. ns- $p > 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$ compared to normal control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to toxic control

Table 4: Effect of Silymarin and UNLE on GSH, LPO, SOD and CAT in TAA Induced Hepatic Injury

Groups	Treatment	GSH (Abs at 412 nm)	LPO (Abs at 535 nm)	SOD (Abs at 560 nm)	CAT (Abs at 620 nm)
Normal control	Vehicle 1 ml / kg	0.65 ± 0.05	0.05 ± 0.02	0.97 ± 0.12	0.54 ± 0.02
Toxic control	Thioacetamide 100 mg / kg	0.26 ± 0.02$^{###}$	0.32 ± 0.03$^{###}$	0.06 ± 0.03$^{###}$	0.22 ± 0.01$^{###}$
Standard	Silymarin 100 mg / kg	0.51 ± 0.04*** (+ 96.82)	0.12 ± 0.01** (- 61.13)	0.73 ± 0.06*** (+ 91.44)	0.41 ± 0.03*** (+ 82.50)
Low dose	UNLE 200 mg / kg	0.45 ± 0.02** (+ 74.00)	0.17 ± 0.05* (- 44.69)	0.45 ± 0.02 ** (+ 83.84)	0.37 ± 0.01** (+ 68.71)
High dose	UNLE 400 mg / kg	0.49 ± 0.03*** (+ 89.60)	0.144 ± 0.01** (- 54.89)	0.55 ± 0.04 *** (+ 88.54)	0.40 ± 0.02** (+ 78.13)

All the values are in absorbance Mean ± SEM, % increase (+) or decrease (-) shown in parentheses, One way ANOVA followed by Tukey's multiple comparison test. ns- $p > 0.05$, $^{###}p < 0.001$ compared to normal control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to toxic control

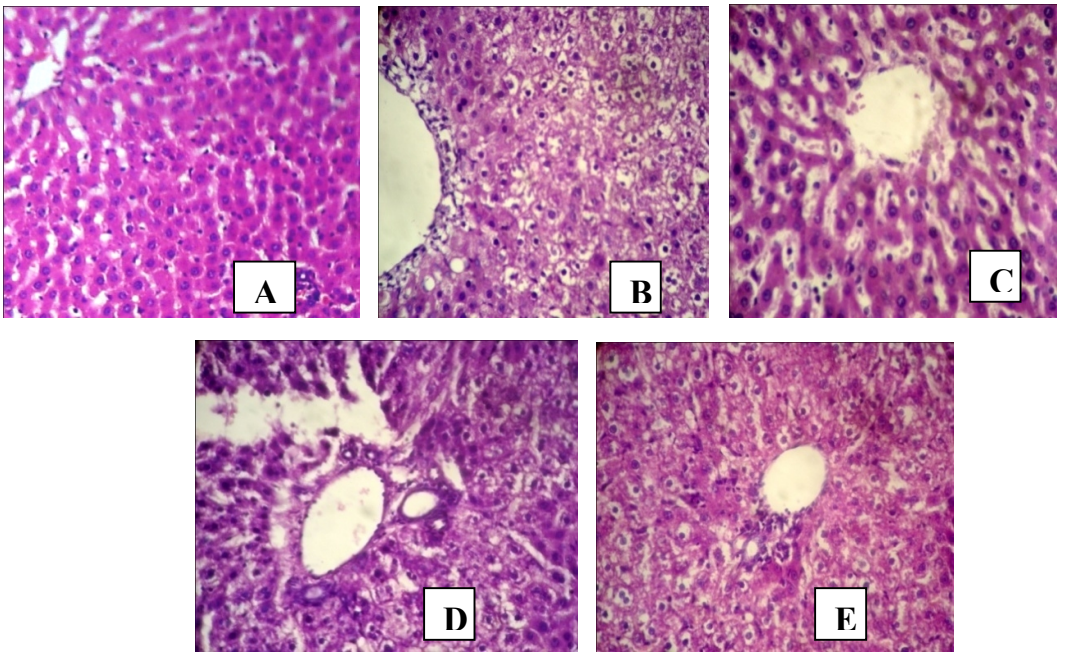

Figure 1: Haematoxylin and Eosin (HandE) Stained Section of Liver in TAA Induced Hepatic Injury. Photographed at Magnification 40X
A) Normal control, B) Positive control: TAA treated 100 mg / kg s.c., C) Standard Silymarin 100 mg / kg + TAA,
D) Low dose 200 mg / kg + TAA, E) High dose 400 mg / kg + TAA

Histopathological Studies

For histopathological study, the fresh liver tissues were collected and immediately fixed in 10 % formalin, dehydrated in gradual ethanol (50-100 % v/v), cleared in xylene and embedded in paraffin. Sections (4-5 μm) were prepared and then stained with hematoxylin-eosin dye for photo microscopic observations.[13]

Statistical Analysis

All the results are expressed as Mean ± SEM, the results were analyzed for statistical significance by one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered as statistical significant.

RESULTS AND DISCUSSION

Acute toxicity studies revealed that the UNLE was safe at a dose of 2000 mg / kg hence one tenth (200 mg / kg) of maximum and one fifth (400 mg / kg) of maximum was selected for present study. TAA is a compound endowed with liver damaging and carcinogenic activity. It has been used to induce a model of acute liver injury in rats. Shortly after its administration, thioacetamide is metabolized to acetamide and thioacetamide-s-oxide. The latter binds to tissue macromolecules responsible for the change in cell permeability, increased intracellular concentration of Ca^{++}, increase in nuclear volume and enlargement of nucleoli and inhibits mitochondria activity eventually leading to hepatic necrosis.[14] An indication of hepatic damage is leakage of cellular enzymes into plasma. When liver cell membrane is damaged, a variety of cytosolic enzymes are released into blood stream, their estimation in serum is a useful quantitative marker of the extent and type of hepatocellular damage. Our results reveal that elevation in levels of SGPT and SGOT in TAA intoxicated animals may be interpreted as a result of hepatocytes damage whereas increase in ALP activity and bilirubin levels reflect the pathological alteration in biliary flow[15] where as Silymarin (100 mg / kg) and UNLE treated animals at both doses (200 mg / kg and 400 mg / kg) significantly prevented hepatic damage by attenuating the increase of these enzymes in serum; In dose dependent manner depicted in Table 1. Thioacetamide intoxicated animals showed acute hepatic damage registered decline in plasma protein and albumin levels. These changes may be related to an increased ubiquitin-associated degradation of protein in the hepatocytes induced by TAA toxic stress. Hypoproteinaemia was accompanied by a significant rise in urea-plasma levels, a trend that may be related to an increase of amino acid oxidative metabolism.[16] Decrease in glucose whereas increase in cholesterol and triglycerides levels indicate disturbance in carbohydrate and lipid metabolism.[17] Experimental results reveal that pretreatment with Silymarin (100 mg / kg) and UNLE (200 mg / kg and 400 mg / kg) significantly elevated total protein where as less effect on albumin and glucose levels. In contrast extract at both doses decreased cholesterol, triglycerides and urea levels; In dose dependent fashion, depicted in Table 2. In present study UNLE, dose dependently reversed relative organ weight, which was elevated in thioacetamide toxicated animals. Results are depicted in Table 3. TAA intoxicated animals showed increase in lipid peroxidation due to excessive production of free radicals resulted in the oxidative stress, which leads to damage of macromolecules e.g. lipids, and can induce lipid peroxidation *in-vivo*. Decrease in endogenous antioxidants like SOD, CAT and GSH has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and liver. Catalase decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. Therefore, reduction in the

activity of catalase may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Glutathione is one of the most abundant tripeptide, nonenzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols.[18] Prophylactic treatment with Silymarin (100 mg / kg) and UNLE at both doses (200 and 400 mg / kg) causes a significant increase in hepatic GSH, SOD and CAT activity and thus reduces reactive free radical induced oxidative damage to liver by decreasing LPO levels depicted in Table 4. From comparative histopathological study, the histological observations of the control animals showed normal hepatocytes with well preserved cytoplasm, prominent nucleus, nucleolus and central vein. There was no sign of inflammation or necrosis in these animals (Figure 1a). In animals treated with TAA only, liver sections showed presence of inflammation cells, severe necrosis and ballooning degeneration of hepatocytes were observed (Figure 1b). Pretreatment with Silymarin at 100 mg / kg dose showed few inflammatory cells and mild necrosis compared to toxic control (Figure 1c). Prophylactic treatment with UNLE at both doses (200 and 400 mg / kg) showed less inflammation, and mild to moderate necrosis compared to toxic control (Figure 1d and e).

CONCLUSION

It can be concluded that ethanolic extract of *Uvaria narum* leaves posses hepatoprotective activity and attenuates the hepatotoxic effects of TAA by membrane stabilizing effect and as antioxidant.

ACKNOWLEDGEMENT

The author's are grateful to management and Principal of Srinivas college of Pharmacy, Valachil, Mangalore, India for providing necessary facilities to carry out experiments.

REFERENCES

1. Sapakal VD, Ghadge RV, Adnaik RS, Naikwade NS, Magdum CS. Comparative hepatoprotective activity of Liv-52 and livomycn against carbon tetrachloride–induced hepatic injury in rats. International Journal of Green Pharmacy 2008; 2(2): 79-82. http://dx.doi.org/10.4103/0973-8258.41175
2. Solomon BG, Rao G, Manju Latha YB, K. Srinivas. Antihepatotoxic activity of *smilax china* roots on CCl_4 induced hepatic damage in rats. Int j pharm sci 2012; 4(1): 494-496.
3. Anitha P, Jai kumar S, Devaki T. Hepatoprotective activity of *Andrographis paniculata* on lipid peroxidase, antioxidant enzymes and liver enzymes in thioacetamide intoxicated rats. Discovery pharmacy 2012; 1(1):15-20.
4. Reddy LJ, Jose B, Jalli RD, Gopu S. Evaluation of antibacterial and DPPH radical scavenging activities of the essential oil and leaf extracts of *Uvaria narum* (Dunal) Wall. International Journal of research in pure and Applied Microbiology 2012; 2(2): 13-19.
5. Shirode DS, Jain BB, Kumar M, Setty SR. Hepatoprotective and antioxidant effects of *Albizzia lebbeck* against thioacetamide induced hepatotoxicity in rats. Indian journal of chemical and pharmaceutical sciences 2012; 5:199-204.
6. OECD/OCDE. 425 OECD guidelines for testing of chemicals acute oral toxicity, up and down procedure 2001; 26: 1-26.
7. Roy CK, Kamath JV, Asad M. Hepatoprotective Activity of *Psidium guajava* Linn. Leaf Extract. Indian Journal of Experimental Biology 2006; 44: 305-311. PMid:16629373
8. Niehius WG, Samuelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. J. Biochem 1968; 6: 126-130. http://dx.doi.org/10.1111/j.1432-1033.1968.tb00428.x
9. Ellaman GL. Tissue sulfhyhydryl groups. Arch Biochem Biophy 1959; 82: 70-7. http://dx.doi.org/10.1016/0003-9861(59)90090-6
10. Sinha AK. Colorimetric assay of catalase. Analytical Biochem 1972; 47: 389-94. http://dx.doi.org/10.1016/0003-2697(72)90132-7
11. Kakkar P, Das B, Vishwanatha PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophysics 1984; 21: 130-2. PMid:6490072
12. Murugaian P, V Ramamurthy and N Karmegam. Hepatoprotective activity of *Wedelia calendulacea* L. against acute Hepatotoxicity in Rats, Research Journal of Agiculture and Biological Sciences 2008; 4(6): 685-7.
13. Luna LG. Manual of histology and staining methods of armed forces institute of Pathology, 3rd Ed, New York: Mc Graw Hill Book Co; 1998.
14. Ansil PN, Nitha A, Prabha SP, Wills PJ, Jazaria V, Latha MS. Protective effect of *Amorphophallus campanulatus* (Roxb.) Blume. Tuber against thioacetamide induced oxidative stress in rats. Asian Pacific Journal of Tropical Medicine 2011; 4(11): 870-877. http://dx.doi.org/10.1016/S1995-7645(11)60211-3
15. Jain NK, Singhai AK. Protective effects of *Phyllanthus acidus* (L.) Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pacific Journal of Tropical Medicine 2011; 4(6): 470-474. http://dx.doi.org/10.1016/S1995-7645(11)60128-4
16. Galisteo M, Suarez A, Montilla MD, Utrilla MD, Jimenez J, Gil A, Faus MJ, Navarra MD. Antihepatotoxic Activity of *Rosmarinus tomentosus* in a Model of Acute Hepatic Damage Induced by Thioacetamide. Phytother 2000; 14: 522–526. http://dx.doi.org/10.1002/1099-1573(200011)14:7<522::AID-PTR660>3.0.CO;2-K
17. Atef M. Attar Al. Attenuating Effect of *Ginkgo biloba* Leaves Extract on Liver Fibrosis Induced by Thioacetamide in Mice. Journal of Biomedicine and Biotechnology 2012; (2012): 1-9.
18. Anbarasu C, Rajkapoor B, Bhat KS, Giridharan J, Amunathan AA, Satish K. Protective effect of *Pisonia aculeata* on thioacetamide induced hepatotoxicity in rats. Asian Pac J Trop Biomed 2012; 2(7): 511–515. http://dx.doi.org/10.1016/S2221-1691(12)60087-2

Cite this article as:

Patel Jagir R, Moses Samuel Rajan, Ashok Shenoy, Prima D'souza, K. Gunjan. Attenuating effect of Uvaria narum (Dunal) Wall. leaves on thioacetamide induced hepatic injury. Int. Res. J. Pharm. 2013; 4(8):214-217 http://dx.doi.org/10.7897/2230-8407.04842

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