BJP

Bangladesh Journal of Pharmacology

Research Article

Hepatoprotective activity of *Thymus linearis* against paracetamol and carbon tetrachloride–induced hepatotoxicity in albino mice

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2014; 9: 230-234
ISSN: 1991-0088

Hepatoprotective activity of *Thymus linearis* against paracetamol and carbon tetrachloride-induced hepatotoxicity in albino mice

Alamgeer[1], Mehwish Nawaz[1], Taseer Ahmad[2], Muhammad Naveed Mushtaq[1] and Asma Batool[1]

[1]Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan; [2]Shifa College of Pharmaceutical Sciences, Shifa Tameer-e-Millat University, Islamabad, Pakistan.

Article Info

Received: 12 March 2014
Accepted: 14 April 2014
Available Online: 17 May 2014

DOI: 10.3329/bjp.v9i2.18329

Cite this article:
Alamgeer, Nawaz M, Ahmad T, Mushtaq MN, Batool A. Hepatoprotective activity of *Thymus linearis* against paracetamol and carbon tetrachloride-induced hepatotoxicity in albino mice. Bangladesh J Pharmacol. 2014; 9: 230-34.

Abstract

In the present study, the hepatoprotective activity of aqueous and ether extracts of *Thymus linearis* (250 and 500 mg/kg orally) was evaluated against carbon tetrachloride- and paracetamol-induced hepatic damage in mice. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assessed. Antioxidant activity of both the extracts was also determined using 1-1-diphenyl-2-picryl hydrazine (DPPH) scavenging method. The results indicated that both the extracts significantly produce a dose dependent reduction in serum levels of ALT, AST, and ALP when compared to carbon tetrachloride- and paracetamol-treated groups. The maximum effect in all the parameters was observed at a dose of 500 mg/kg. The extracts also demonstrated a significant anti-oxidant activity. LD_{50} of both extracts was found to be 1050 and 900 mg/kg respectively. It is conceivable that the hepatoprotective activity of *T. linearis* might be due to the presence of certain pharmacologically active compounds.

Introduction

Many synthetic drugs are used to treat liver diseases but unfortunately these drugs have serious side effects (Guntupalli et al., 2006). The role of traditional medicines in solving health problems is valuable globally. This is even more impressive considering the fact that about 80% of people living in less developed countries rely exclusively on traditional medicines for their health care needs. Natural plants have a very important role in treatment of liver diseases. There are many natural plants in world which have hepatoprotective activity (Gite et al., 2010) like *Carissa spinarum* (Hegde and Joshi, 2010), *Cocculus hirsutus* (Thakare et al., 2009), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), *Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala* (Akhtar et al., 2013), *Ipomoea staphylina* (Bag and Mumtaz, 2013), *Suaeda fruticosa* (Rehman et al., 2013), *Trianthema decandra* (Balamurugan and Muthusamy, 2008) and *Trichodesma sedgwickianum* (Saboo et al., 2013) showed hepatoprotective effect.

Pakistan is granted with large number of medicinally important plants and has ancient herbal treatment method. The local communities of different regions of Pakistan have centuries old knowledge about traditional uses of plants occurring in their areas. Several indigenous plants are used to treat liver disorders (Ivvora et al., 1989).

Thymus linearis Benth belongs to the Labiatae family which is one of the largest and most diverse family of medicinal plants. It is mostly used in home remedies. Plant is aromatic, antiseptic, diaphoretic, analgesic, diuretic, carminative and stimulant. It is also used in rheumatism gout and sciatica. The herbal decoction may assist to prevent hair loss (Sharma et al., 2005). It is also given in weak vision, complaints of liver, suppression of urine and mensturation (Qureshi, 2007).

Hence, this study was undertaken to investigate the hepatoprotective effects of extract on paracetamol and carbon tetrachloride (CCl_4)-induced albino mice hepatic injury.

Material and Methods

Chemicals

CCl_4 (Merck Darmstadt, Germany), paracetamol (Zenith Pharma Karachi, Pakistan), silymarin, diagnostic kits for the estimation of ALT, AST and ALP (Diasys, Germany) were used. Chemicals and reagents used in these experiments were of analytical grade and prepared in distilled water.

Plant material used

The selected plant was collected from the mountains of the village Shikiyote; district Gilgit, Gilgit-Baltistan. Plant was identified and authenticated by Dr. Shair Wali, Department of Botany, Karakoram International University, Gilgit-Baltistan. Plant material was shade dried and powdered with a Chinese herbal grinder. The powdered material was stored in well closed cellophane bags at 4°C in refrigerator.

Preparation of plant extracts

The powdered plant was extracted by method of cold maceration. One kg of plant powder was extracted by using solvents; ether and distilled water (Aghel et al., 2011). For extraction with each solvent, the plant powder was soaked for 72 hours with occasional shaking. It was passed through muslin cloth and then filtered through filter paper. The aqueous and ether extracts were prepared with the help of rotary evaporator.

Animals used

Adult healthy albino mice (25-30 g) of either sex were used in the study and kept under natural light and dark cycle at a temperature of 28 ± 4°C, given a standard pellet diet and water *ad libitum*.

Hepatoprotective activity against paracetamol-induced toxicity in mice

After an overnight fasting, mice were divided into seven groups of five animals each. Group 1 was control group which received single dose of 5% tween 80 daily for 4 days orally and on 3rd day single dose of 40% sucrose solution. Group 2 was paracetamol group and it received single dose of 5% tween 80 daily for 4 days orally and on 3rd day single dose of paracetamol suspension (250 mg/10 mL water). Group 3 and 4 were treated daily 250 and 500 mg/kg of ether extract, while Group 5 and 6 received 250 and 500 mg/kg of aqueous extract of *T. linearis* respectively for 4 days orally and on 3rd day single dose of paracetamol suspension. Group 7 served as silymarin treated which received silymarin for four days and on 3rd day single dose of paracetamol. Animals were sacrificed on 5th day, and their blood was collected for biochemical study (Maheswari et al., 2008).

Hepatoprotective activity in CCl_4-induced toxicity in mice

After an overnight fasting, mice were divided into seven groups of five mice each. Group 1 served as negative control and was treated with normal saline (0.9%) for four days. Group 2 was treated with normal saline and CCl_4, on 1st and 4th day, and on 2nd and 3rd day respectively. Group 3 and 4 were treated daily 250 and 500 mg/kg of ether extract, while group 5 and 6 received 250 and 500 mg/kg of aqueous extract of *T. linearis* respectively for 4 days orally and on the 2nd and 3rd day CCl_4 was given half an hour after the administration of extract dose. Group 7 served as silymarin treated and was given CCl_4 and silymarin on 1st and 4th day, and on 2nd and 3rd day respectively. Animals were sacrificed on 5th day, and their blood was collected for biochemical tests (Aghel et al., 2011).

Anti-oxidant activity

Solution of 1-1-diphenyl-2picryl hydrazine (DPPH) was prepared by dissolving 4.3 mg of DPPH in 3.3 mL of methanol and covered with aluminum foil to protect from light. 150 µL DPPH solution was added to 3 mL of methanol and its absorbance was measured at 516 nm as control reading. Different concentrations (10, 20, 40, 60, 80, 100 µg/mL) of aqueous and ether extracts of plant were made and the volume was made uniformly to 150 µL using methanol. Each of the samples was then further diluted with methanol up to 3 mL and to each, 150 µL DPPH solution was added. After 15 min absorbance was measured at 516 nm by using methanol as blank at UV visible spectrometer. The capability to scavenge the DPPH radicals was calculated using the following formula:

$$\text{DPPH scavenged (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Where, $A_{control}$ = The absorbance of the control reaction mixture and A_{Sample} = the absorbance of sample at different concentrations (Molyneux, 2004)

Acute toxicity test

The aim of this experiment was to determine the LD_{50} of crude extract. Adult healthy mice of either sex, weighing 25-30 g were randomly divided into nine groups of 2 animals each.

Group 1 served as control and received normal saline 10 mL/kg. Group 2, 3, 4, 5, 6, 7, 8 and 9 were given different doses of aqueous and ether extracts of *T. linearis* in an ascending order i.e.100, 300, 600, 900 mg/kg respectively. The mortality rate was observed for 24 hours. Since no mortality occurred so another nine groups of mice were taken. They were again treated with various doses of aqueous and ether extracts in an ascending order i.e. 1200, 1500, 1800, 2000 mg/kg respectively. All the doses were administered by

Table I

Effects of ether and aqueous extracts of *Thymus linearis* and silymarin on liver enzymes against paracetamol-treated hepatotoxic albino mice

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	37.4 ± 7.2	30.6 ± 5.3	74.0 ± 6.2
Paracetamol control	96.4 ± 9.7	74.2 ± 7.5	100.2 ± 6.1
Ether extract (250 mg/kg) + Paracetamol	76.6 ± 3.2[a]	49.8 ± 3.1[a]	119.8 ± 17.1[a]
Ether extract (500 mg/kg) + Paracetamol	65.2 ± 2.2[a]	41.2 ± 5.3[a]	106.4 ± 3.3[a]
Aqueous extract (250 mg/kg) + Paracetamol	82.4 ± 9.1[b]	52.0 ± 2.3[b]	82.4 ± 1.7[b]
Aqueous extract (500 mg/kg) + Paracetamol	73.2 ± 6.1[b]	49.2 ± 3.4[b]	78.0 ± 0.8[b]
Silymarin + Paracetamol	64.4 ± 1.3[c]	44.0 ± 2.4[c]	69.4 ± 1.2[c]

Data are expressed as mean ± SEM; Where, [a]p<0.05, [b]p<0.01 and [c]p<0.001, as compared to paracetamol control group

Table II

Effects of EETLB, AETLB and silymarin on liver enzymes against CCl_4 treated hepatotoxic Albino mice albino mice

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	37.4 ± 7.2	30.6 ± 5.1	74.0 ± 6.2
CCl_4 control	127.4 ± 12.8	66.2 ± 7.8	131.6 ± 13.6
Ether extract (250 mg/kg)+ CCl_4	100 ± 3.9[a]	51.4 ± 3.8[c]	96.2 ± 1.7[a]
Ether extract (500 mg/kg)+ CCl_4	76.6 ± 1.7[c]	43.4 ± 5.2[c]	92.0 ± 3.1[a]
Aqueous extract (250 mg/kg)+ CCl_4	99.4 ± 5.8[a]	52.2 ± 3.8[a]	111.6 ± 7.6[a]
Aqueous extract (500 mg/kg)+ CCl_4	65.8 ± 11.0[c]	39.4 ± 3.4[a]	104.8 ± 2.5[b]
Silymarin + CCl_4	32.6 ± 2.3[c]	34.0 ± 2.3[c]	84.8 ± 1.9[c]

Data are expressed as mean ± SEM; Where, [a]p<0.05, [b]p<0.01 and [c]p<0.001 as compared to CCl_4 treated group

intraperitoneal route. The highest dose which did not kill any mice, and the lowest dose which killed only one mice was noted. LD_{50} was calculated from the mean of these two doses (Shetty et al., 2007).

Preliminary phytochemical analysis

The aqueous and ether extracts of *T. linearis* were analyzed for the presence of different phytochemical constituents such as flavonoids, reducing sugars, tannins, phenolic compounds, saponins, alkaloids and cardiac glycosides by using standard methods (Khandelwal, 2006).

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM) and analyzed by using t-test. P<0.05 was considered as significant.

Results

There was a significant increase in the level of liver enzymes in paracetamol-treated group compared to normal control group. Ether extract significantly (p<0.05) decreased the level of liver enzymes in 250 mg/kg and 500 mg/kg extract treated group. Aqueous extract also significantly (p<0.01) decreased the level of liver enzymes in 250 mg/kg and 500 mg/kg treated group. There was highly significant (p<0.001) decrease in the liver enzymes (ALT, AST, ALP) in silymarin-treated group compared to paracetamol treated group (Table I).

In extract treated group with the dose of 250 mg/kg there was a significant (p<0.05) decrease in liver enzymes and with the dose of 500 mg/kg there was a significant (p<0.001), (p<0.05), (p< 0.01) decrease in the level of ALT, AST and ALP respectively. However, there was highly significant (p<0.001) decrease in the liver enzymes in silymarin-treated group compared to CCl_4 -treated group (Table II).

In CCl_4 treated group there was a significant increase in the level of liver enzymes compared to normal control group. Ether extract with a dose of 250 mg/kg significantly (p<0.05), (p<0.001), (p<0.05) decreased the level of ALT, AST, ALP respectively. There was a highly significant decrease in the liver enzymes in 500 mg/kg and silymarin-treated group compared to CCl_4-treated group (Table II).

Aqueous extract of *T. linearis* in a concentration of 10, 20, 40, 60, 80 and 100 µg/mL significantly scavenged 84.0, 88.2, 89.9, 93.4, 94.9, 95% DPPH free radical respectively. While ether extract significantly 85.7, 88.9, 91.1, 94.7, 95.7, 96.1% scavenged DPPH free radical in a concentration-dependent manner. Results obtained were comparable with standard anti-oxidant ascorbic acid (Figure 1).

LD_{50} of ether and aqueous extracts of *Thymus linearis* was found to be 1050 and 900 mg/kg in mice respectively.

Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids. The other secondary metabolites like tannins, saponins, steroids, cardiac gly-

Table III

Preliminary phytochemical analysis of *Thymus linearis*

	*A*queous extract	*E*ther extract
Tanins	+	+
Saponins	-	+
Flavanoids	+	+
Steroids	+	-
Cardiac glycosides	+	+
Reducing sugar	+	-
Indole alkaloids	+	+
Anthraquinone glycosides	-	-
Terpenoids	+	+

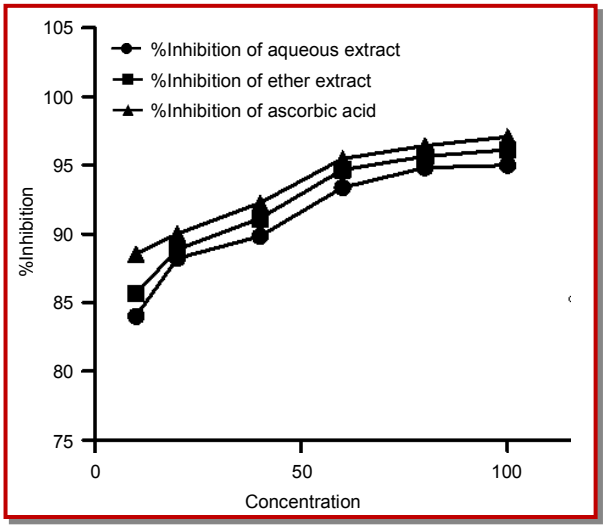

Figure 1: Percent inhibition of ascorbic acid, aqueous and ether extract of *Thymus linearis*

cosides, and reducing sugar were also present (Table III).

Discussion

The use of medicinal plants has been increased during the last few decades. However, the use of these medicinal plants into the modern therapy requires their toxicological and pharmacological evaluation on scientific basis. So, the current study was carried out to evaluate the hepatoprotective effect of *T. linearis* in paracetamol- and CCl_4-induced hepatotoxic mice. In the present study aqueous and ether extract of the plant showed significant hepatoprotective effect. The results were in agreement with the previous study (Ahsan et al., 2009).

In our current study, the levels of ALT, AST and ALP were increased in paracetamol and treated mice which is in accordance with the previous findings (Mittal et al., 2010). All these enzymes metabolize in liver and damage to the liver causes the leakage of these enzymes into the blood circulation and their level increases (Wakchaure et al., 2011).

Treatment with *T. linearis* extracts restored the elevated values of liver enzymes near to normal level, which is a clear manifestation of antihepatotoxic effect of *T. linearis* extracts. The hepatoprotective activity of the extracts may be due to their stabilizing effect on plasma membrane as reported earlier by Nadeem et al. (2011). The inhibition of lipid peroxidation by the active constituents of the extracts might also be involved in hepatoprotective effect which is duly supported by the study of Kanchana et al. (2011). The oxidative stress is considered an important cause of liver injury, induced by hepatotoxic agents. The free radicals especially reactive oxygen species (ROS) are involved in hepatic tissue damage. In our study, aqueous and ether extracts demonstrated the significant anti-oxidant activity in DPPH scavenging assay which is compared with standard anti-oxidant ascorbic acid. The extracts may produce the hepatoprotective effect by neutralizing the effect of free radical species. The results are in complete agreement with the investigation of Pourmorad et al. (2006). Increased protein synthesis, improved lipoprotein metabolism and presence of phytochemical constituents may also be related to the hepatoprotective effect of extracts (Ahsan et al., 2009).

Extracts in a dose of 500 mg/kg demonstrated more significant effects as compared to 250 mg/kg dose. So the aqueous and ether extracts produced the hepatoprotective effect in dose-dependent manner. Silymarin is used as standard hepatoprotective agent and has protective effect on hepatocytes (Kshirsagar et al., 2011). Aqueous and ether extracts of *T. linearis* attenuated significantly the decreased level of these enzymes and caused a subsequent recovery towards normalization almost like that of silymarin treatment. This study calls for further activity-oriented fractionation of these extracts to isolate the active principle (s) and elucidate exact mechanism of action.

Conclusion

The aqueous and ether extract of *T. linearis* have dose-dependent hepatoprotective activity and it may be due to the presence of active constituent(s) in the extracts.

Ethical Issue

The study protocol was approved by the Institutional Animal Ethics Committee (IEC) Faculty of Pharmacy, University of Sargodha (Approval No. 25-A12 IEC UOS). All the experiments performed complied with the rulings of National Research Council (NRC, 1996).

References

Aghel N, Kalantari H, Rezazadeh S. Hepatoprotective effect of *Ficus carica* leaf extract on mice intoxicated with carbon tetrachloride. Irani J Pharm Sci. 2011; 10: 63-68.

Ahsan R, Islam KM, Musaddik A, Haque E. Hepatoprotective activity of methanol extract of some medicinal plants against carbontetrachloride induced toxicity in albino rats. Global J Pharmacol. 2009; 3: 116-22.

Akhtar MS, Asjad HMM, Bashir S, Malik A, Khalid R, Gulzar F, Irshad N. Evaluation of anti-oxidant and hepatoprotective effects of Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala (KGA). Bangladesh J Pharmacol. 2013; 8: 44-48.

Akhtar MS, Nadeem M, Rashid HU, Bashir S. Hypoglycemic activity of different fractions of *Berberis aristata* root-bark in normal and alloxan diabetic rabbits. Can J of App Sci. 2011; 1: 16-28.

Ali M, Qadir MI, Saleem M, Janbaz KH, Gul H, Hussain L, Ahmad B. Hepatoprotective potential of *Convolvulus arvensis* against paracetamol-induced hepatotoxicity. Bangladesh J Pharmacol. 2013; 8: 300-04.

Bag AK, Mumtaz SMF. Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylina* leaves. Bangladesh J Pharmacol. 2013; 8: 263-68.

Balamurugan G, Muthusamy P. Observation of the hepatoprotective and anti-oxidant activities of *Trianthema decandra* Linn. (Vallaisharunnai) roots on carbon tetrachloride-treated rats. Bangladesh J Pharmacol. 2008; 3: 83-89.

Guntupalli M. Hepatoprotective effects of rubiadin, a major constituent of Rubia Cordifolia. J Ethnopharmacol. 2006; 103: 484-90.

Hegde K, Joshi AB. Hepatoprotective and anti-oxidant effect of *Carissa spinarum*root extract against CCl_4 and paracetamol-induced hepatic damage in rats. Bangladesh J Pharmacol. 2010; 5: 73-76.

Ivorra S, Paya M, Villar A. A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol. 1989; 27: 243-75.

Kanchana N, Sadiq AM. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol-induced liver toxicity in rats. Int J Pharm Pham Sci. 2011; 3: 151-54.

Khandelwal KR. Practical pharmacognosy. 12th ed. Pune, Nirali Prakashan Publishers, 2006, 149-53.

Khan AZ, Mohammad A, Iqbal Z, Anis I, Shah MR, Nadeem S, Rabnawaz M, Shahidullah A, Khan H, Khan I. Molecular docking of viscosine as a new lipoxygenase inhibitor isolated from *Dodonaea viscose*. Bangladesh J Pharmacol. 2013; 8: 36-39.

Kshirsagar AD, Mohite R, Aggrawal AS, Suralkar UR. Hepatoprotective medicinal plants of Ayurveda: A review. Asian J Pharmaceut Clin Res. 2011; 4: 1-8.

Maheswari C, Maryammal R, Venkatanarayanan S. Hepatoprotective activity of *Orhosiphon stamineus* on liver damage caused by paracetamol in rats. JJBS. 2008; 1: 105-08.

Mittal DK, Joshi D, Shukla S. Protective effects of *Polygonum bistorta* (Linn.) and its active principle against acetaminophen-induced toxicity in rats. Asian J Exp Biol Sci. 2010; 1: 951-58.

Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TPA. Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr. 2007; 40: 163-73.

Molyneux PH. The use of stable free radical diphenyl picrylhydrazyl (DPPH) for estimating anti-oxidant activity. Songklanakarin J Sci Tech. 2004; 26: 211-19.

Nadeem MPC, Dandiya PC, Pasha M, Imran D, Balani K, Vohora SB. Hepatoprotective activity of *Solanum nigrum* fruits. Fitoterapia 1997; 68: 245-51.

NRC. Guide for the care and use of laboratory animals. Washington DC, National Academy Press, 1996.

Periasamy M, Pavankumar K, Gangadhar V, Jeeva T, Anandhan R, Sengottuvelu S. Hepatoprotective and anti-oxidant activity of *Euphorbia ligularia* against carbon tetrachloride induced hepatotoxicity in Wistar rats. Int J Res Pharm Biomed Sci. 2012; 3: 100-04.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Anti-oxidant activity, phenol and flavonoids contents of some selected Iranian medicinal plants. Afr J Biotech. 2006; 5: 42-1145.

Qureshi LR. Ethnobotanical studies of selected medicinal plants of Sudhan Gali and Ganga Chotti Hills, District, Azad Kashmir. Pak J Bot. 2007; 39: 2275-83.

Rajesh MG, Latha MS. Hepatoprotection by *Elephatopus scaber* Linn in CCl_4 induced liver injury. Indian J Physiol Pharmacol. 2001; 45: 481-86.

Rehman JU, Saqib NU, Akhtar N, Jamshaid M, Asif HM, Sultana S, Rehman RU. Hepatoprotective activity of aqueous-methanolic extract of *Suaeda fruticosa* in paracetamol-induced hepatotoxicity in rabbits. Bangladesh J Pharmacol. 2013; 8: 378-81.

Saboo SS, Tapadiya G, Farooqui IA, Khadabadi SS. Free radical scavenging, *in vivo* antioxidant and hepatoprotective activity of folk medicine *Trichodesma sedgwickianum*. Bangladesh J Pharmacol. 2013; 8: 58-64.

Sharma PK, Lal B. Ethnobotanical notes on some medicinal and aromatic plants of Himachal Pradesh. Indian J Traditional Knowledge. 2005; 4: 424-28.

Shetty A, Shyamjith, Deepa, Alwar, MC. Acute toxicity studies and determination of median lethal dose. Current Sci. 2007; 93: 917-20.

Wakchaure D, Jain D, Singhai AK, SomanI R. Hepatoprotective activity of *Symplocos racemosa* Roxb. bark extract in carbon tetrachloride induced liver damage in rats. J Ayurveda Integr Med. 2011; 2: 137-43.

Author Info
Taseer Ahmad (Principal contact)
e-mail: drtasir2011@ gmail.com