Online : ISSN 2349-669X
Print : ISSN 0973-9874

J.Pharm.Chem

CODEN: JPCOCM

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)
Indexed in Chemical Abstract and Index Copernicus (IC Value 5.28)

www.stfindia.com
www.jpc.stfindia.com

Online : ISSN 2349-669X
Print : ISSN 0973-9874

J.Pharm.Chem

CODEN: JPCOCM

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)

Volume 10 • Issue 4 • October – December 2016

CONTENTS

Evaluation of Acute Toxicity and Hepatoprotective Activity of the Methanolic Extract of *Hibiscus Radiatus* Cav. Leaves on Liver Damage Caused By Carbon Tetrachloride in Rats..... 3
MOHAMMAD SHAMIM QURESHI[1*], A. VENKATESHWAR REDDY[2] AND G. S. KUMAR[3]

Instruction to Authors

✦

VIEWS

The views and opinions expressed in this journal are those of the contributors; Science-Tech Foundation does not necessarily concur with the same. All correspondence should be addressed to the Editor-In-Chief (Hon.), Journal of Pharmacy and Chemistry (Science-Tech Foundation), Plot No 22, Vidyut Nagar, Anantapur - 515 001, Andhra Pradesh, India. • e-mail:editorjpc@gmail.com. **Send your queries at www.jpc.stfindia.com, www.stfindia.com**

Online : ISSN 2349-669X
Print : ISSN 0973-9874

Evaluation of Acute Toxicity and Hepatoprotective Activity of the Methanolic Extract of *Hibiscus Radiatus* Cav. Leaves on Liver Damage Caused By Carbon Tetrachloride in Rats

MOHAMMAD SHAMIM QURESHI[1*], A. VENKATESHWAR REDDY[2] AND G. S. KUMAR[3]

[1]Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Hyderabad, Telangana, India.
[2]Department of Pharmacology, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana, India.
[3]Department of Life Sciences, School Of Pharmacy, International Medical University, Bukit Jallil, Kuala Lumpur, Malaysia.

ABSTRACT

The present study observed the potential hepatoprotective action of methanolic extract of *Hibiscus radiatus*leaf (MEHR) on carbon tetrachloride-induced liver damaged in rats. Thirty rats divided into five groups i.e.; group 1 – control; group 2 – CCl_4 induced; group 3 – CCl_4 + Silymarin 100 mg/kg;group 4 – CCl_4 + MEHR 200 mg/kg; group 5 – CCl_4 + MEHR400 mg/kg of six rats in each were used. Administration of CCl_4 induced hepatic damage in rats, as evidenced by a significant ($p<0.05$) increase in the levels of AST, ALT, cholesterol and bilirubin levels. However, administration of methanolic extract of *Hibiscus radiatus*(200 and 400mg/kg) after CCl_4administration (group 4 and 5) brought a significant ($p<0.05$) reduction in values of these parameters compared to the CCl_4 treated group (group 2). The potential hepatoprotective activity of the extract was also demonstrated by its regenerative action on some damaged liver tissues, as evidenced by the histopathological studies of the representative liver sample of group 5 rats' liver section, which showed hepatic regeneration, with no visible pathology. This study therefore showed the potential of the *Hibiscus radiatus*leaf extract to decrease the levels of serum markers enzymes, indicating the protection of hepatic cells, and to confer some stage of protection against CCl_4 induced hepatocellular injury.

Key words: CCL_4, Methanol, Extract, Hepatoprotective, Silymarin.

Introduction

The liver plays a vital role in metabolism and has some physiological functions [1]. Liver detoxification occurs in two phases, namely phase I (involving oxidation, reduction, and hydrolysis) and phase II (involving synthetic conjugations with sulfates, glucuronic acid, glutathione, acetate, and glycine), and these phases convert toxic materials into harmless metabolites and then excrete them from the body [2]. Liver diseases are considered serious problems which can be caused by toxic chemicals, drugs, and virus infiltration through ingestion or infection [3]. These toxins induce the production of reactive oxygen species (ROS), which can attack hepatic tissue and cause serious injury [4,5].

Medicinal plants with hepatoprotective activity contain a large number of bioactive molecules. The identification of these molecules contained in a biomass complex requires careful selection and execution of appropriate bioassays during the various stages of the research process [6].One hepatoprotective agent used widely in the treatment of various liver disorders, such as hepatitis or fatty infiltration caused by alcohol or toxins, is the standardized extract of *Silybummarianum*, known as milk thistle or silymarin (SLM) [7-10]. It is a complex mixture of the flavonolignanssilybinin (SLB), silychristin, silydianin, and isosilybin. SLB, a polyphenolic molecule, is the major component of SLM and is responsible for its pharmacological activity [11,12]. SLM is poorly absorbed, although the bioavailability of SLB is higher than that of phosphatidylcholine (SLP) [13,14].The major inducers of hepatic damage used when evaluating hepatoprotective activity are carbon tetrachloride (CCl_4) and paracetamol (acetaminophen, APAP). However, there are few reports on their use and in vitro characteristics [15-17].

The mechanisms responsible for the in vivo liver toxicity of both compounds are complex and involve several

**Address for correspondence: sports78@gmail.com*

cell types [18,19]. CCl_4 undergoes metabolic activation in a cytochrome P-450-dependent step to produce free radicals, which can initiate lipid peroxidation. The toxicity induced by CCl_4 in vivo and in cultured hepatocytes involves stimulation of lipid peroxidation, which is detected as an increase in malondialdehyde (MDA) formation [20].APAP is metabolized mainly in the liver to excretableglucuronide and sulfate conjugates. However, the hepatotoxicity of APAP has been attributed to the formation of toxic metabolites, which occurs when APAP is activated by hepatic cytochrome P-450 [21] to a highly reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI) [22]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid. However, when its rate of formation exceeds the rate of detoxification by GSH, NAPQI oxidizes tissue macromolecules such as lipids and —SH group proteins, and calcium homeostasis is altered by depletion of GSH [23].

Hibiscus radiatus Cav. also known asmonarch rosemallow, is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fibre. The genus Hibiscus (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees[24].The plant is about 3m tall and has a deep penetrating taproot. It has a smooth or nearly smooth, cylindrical, typically dark green to red stems. Leaves are alternate, 7 to 11cm long, green with reddish veins and long and short petioles. Leaves of young seedlings and upper leaves of older plants as simple; lower leaves are deeply 3 to 5 or even 7 – lobed and the margins are toothed[25].This study investigated the hepatoprotective effect of the methanol extract of *Hibiscus radiatus* leaves on CCl_4-induced liver damage in rats. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were estimated to evaluate liver function.

MATERIALS AND METHODS

Collection of plant material

The plant *Hibiscus radiatus*Cav. belonging to the family *Malvaceae* were collected from local area of Basna, district - Mahasamund, Chhattisgarh, India and was identified and authentified by M. Ahmedullah scientist 'E' Botanical Survey of India (BSI), Deccan Regional Centre, Hyderabad 500048, Telangana (Establishment under the Ministry of Environment & Forests, Government of India). The plant voucher No. is BSI/DRC/2015-16/Tech./664/07 dated 30-10-2015.

Drugs and Chemicals

All the chemicals were analytical grade. CCl_4 was obtained from pharmacognosy and phytochemistry lab, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad. India. Silymarin was obtained from Allied Chemicals & Pharmaceuticals (P) Ltd. New Delhi. It is a poly herbal formulation which produces hepatoprotective activity against CCl_4.

Preparation of the Extracts

The leaves of *Hibiscus radiatus*were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by hot percolation method using petroleum ether, chloroform, methanol, and distilled water in a soxhlet extractor[26].The different extracts obtained were evaporated using a rotary evaporator to obtained semisolid mass. The extracts thus obtained were subjected to phytochemical screening and the methanolic extract of*Hibiscus radiatus*(MEHR) and aqueous extract of *Hibiscus radiatus* (AEHR) was used for further studies.

Ethical Committee Approval

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) No.-IAEC/AUCOP/2016/01, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad, Telangana, India. The experimental animals were treated as per the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Phytochemical Analysis

The qualitative chemical tests carried out for the identification of the different phytoconstituents present in the powered crude drugs by standard procedures. They are usually tested for the presence of alkaloids, flavonoids, phenols and phenolic compounds, tannins, glycosides, triterpenes, steroids, saponins etc. [27,28].

Acute Oral Toxicity

Acute oral toxicity study was performed as per OECD-423 guidelines category IV (acute toxic class method,). Albino rats (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were kept fasting for 4 h with free access to water only. MEHR was administered orally with maximum dose of 2000 mg /kg body weight by gastric intubation. The mortality was observed for three days. If mortality was observed in 2 out of 3 animals or 3 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed then the procedure was repeated for further higher dose such as 3000 mg/kg of body weight[29].

HEPATOPROTECTIVE ACTIVITY

Animal Model

Healthy Wistar Albino Rats weighing about (150-250gm) of either sex was used for the studies. The animals were housed in large polypropylene cages in a temperature controlled room (25˚C ±3˚C), relative humidity (50±20 %) and provided with standardized pellet feed and clean drinking water *ad libitum*.

CCl_4 induced-hepatotoxic activity

The animals were randomly divided into 5 groups of 6 animals per group[30]. Group 1 received 1 ml of 30 % PEG orally as a control group, Group 2 received 1 mL/kg body weight of CCl_4 subcutaneously for 7 days as a toxic group, Group 3 received Silymarin (100mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) of body weight for 7 days,Group 4 received MEHR(200mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days,Group 5 received MEHR (400mg/kg, p.o.) and CCl_4 (1mL/kg, s.c.) for 7 days. All rats were sacrificed by cervical dislocation 24 h after the last treatment. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anesthesia. Collected blood was allowed to clot and serum was separated at 3500 rpm for 15 min for carrying out further biochemical investigations. One part of liver was dissected out and used for biochemical and histopathological studies[31-34].

Measurement of serum biochemical parameters

The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined using the Hitachi 912 clinical chemistry automatic analyzer (Roche Diagnostic GmbH, Mannheim).

Histopathology

Animals from control and treated groups were used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver.The liver tissue was dissected out and fixed in 10% formalin solution and then dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5–6 mm) were made and then stained with hematoxylin and eosin dye for photomicroscopic observation.

Statistical analysis

The statistical significance were determined by using one way ANOVA followed by Dunnett's multiple comparison test by using Graph p Instat software. The values were represented as Mean ± SEM, (n=6). Less than 0.05 value of P was considered to be statistically significant. *P<0.5 **P<0.01 and ***P<0.001, when compared with control and toxicant group as applicable.

RESULTS

Preliminary Phytochemical studies indicate the presence of flavonoids and phenolics compound were noticed in methanolic extract of *Hibiscus radiatus*leaves. Therefore, there is possibility that methanolic extract of *Hibiscus radiatus*leaves may possess hepatoprotective activity.The results were shown in Table No.1.The extract did not produce any toxic symptoms of mortality up to the dose level of 2000 mg/kg body weight in the treated animals and hence $1/10^{th}$ (200mg/kg.) dose were selected for screening hepatoprotective activity.

Hepatoprotective activity

Histopathological observation of rat liver tissue from the control group (Group 1) showed hepatic cells (normal) with central vein and sinusoidal dilation (Figure 1). In the CCl_4 group (Group 2), severe hepatotoxicity was showed in the form of severe necrosis and disappearance of nuclei (Figure 2). Histopathological analysis showed that the pathological lesions caused by CCl_4 were very minimal in groups pretreated with methanolic extract of *Hibiscus radiatus*(Group 4 and 5). Normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area were observed in groups 4 and 5, treated with methanolic extract of *Hibiscus radiatus*, 200 and 400 mg/kg body weight, respectively (Figures 4 and 5). Liver tissue from CCl_4 + silymarin group (Group 3) had normal hepatic cells with portal vein and portal artery (Figure 3).

Biochemical studies

The effects of methanolic extract of *Hibiscus radiatus* on AST, ALT, ALP, bilirubin, and total protein levels in rats with CCl_4 induced liver damage were summarized in Table 2. Administration of CCl_4 after 18 h resulted in a significant

Table -1

Phytochemical screening of different extracts of *Hibiscus radiatus* leaves

Tested Group	Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
Alkaloids	---	---	+++	+++
Glycosides	---	---	+++	---
.Phenolic com	---	---	+++	+++
Steroids	+++	---	---	---
Saponins	---	---	---	+++
Flavonoids	---	---	+++	+++
Proteins	+++	---	---	---
Carbohydrates	---	---	---	---
Tannins	---	---	+++	---

Note: (+++) Present (---) Absent

elevation of hepatospecific serum markers such as AST, ALT, ALP, bilirubin, and total protein in the CCl_4 group (Group II) in comparison with the control group (Group I). On administration of methanolic extract of *Hibiscus radiatus* (Groups 4 and 5) and CCl_4 + silymarin group (Group 3), the serum markers were restored to the normal levels.

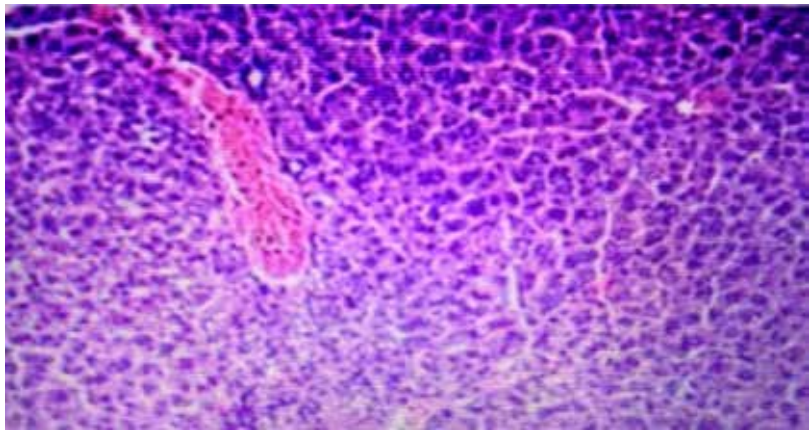


Fig. 1: Liver section of normal rats from Group 1

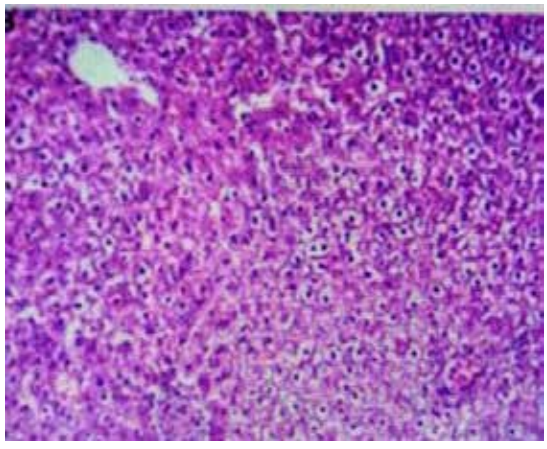


Fig. 2 : Liver sectiontreated with CCl_4 from Group 2

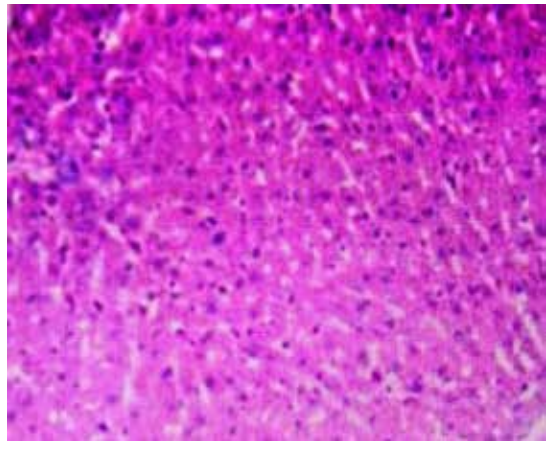


Fig. 3 : Liver sections treated with CCl_4 + Silymarin (100 mg/kg, p.o) from Group 3

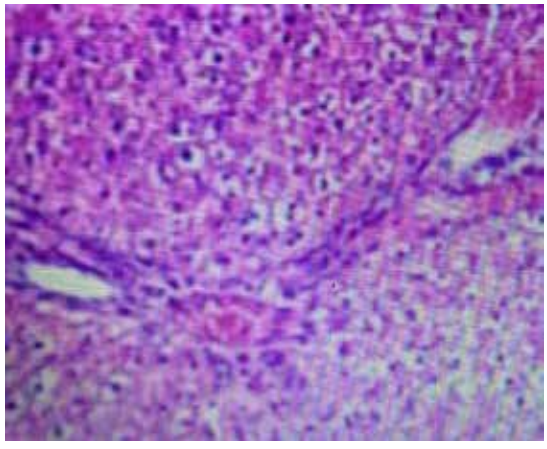


Fig. 4 : Liver sections treated with CCl_4 + MEHR (200 mg/kg, p.o) from Group 4

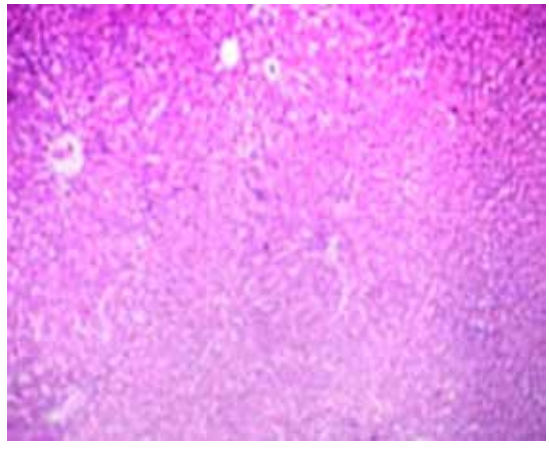
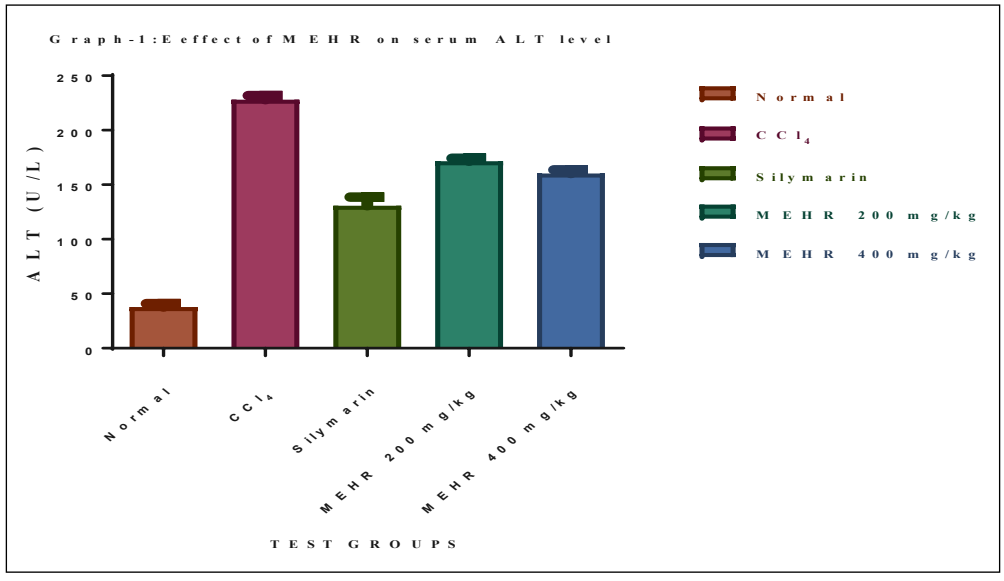
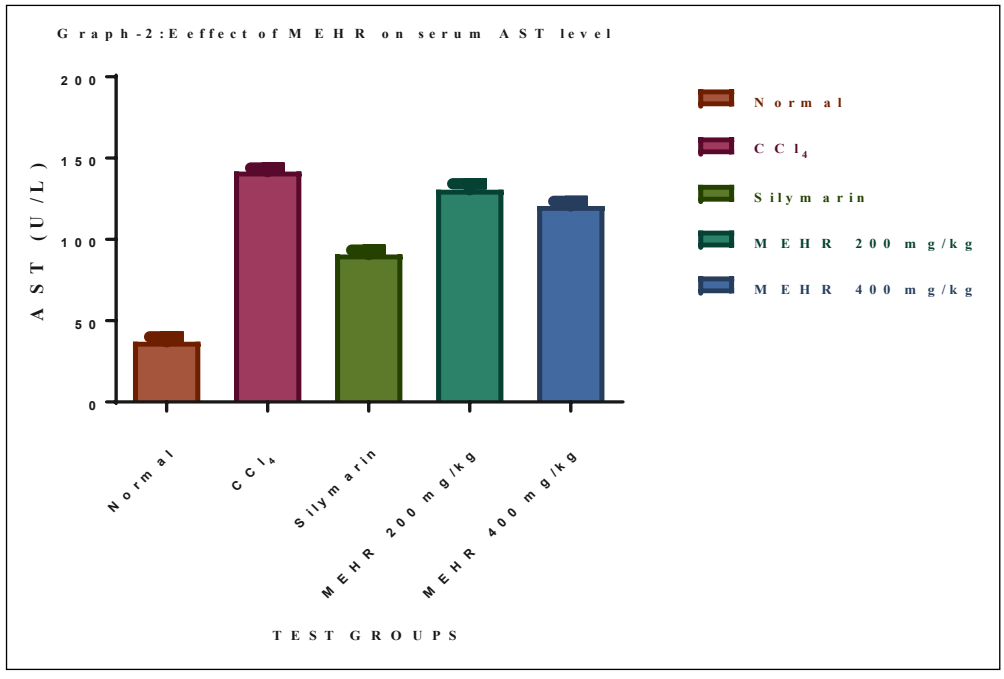
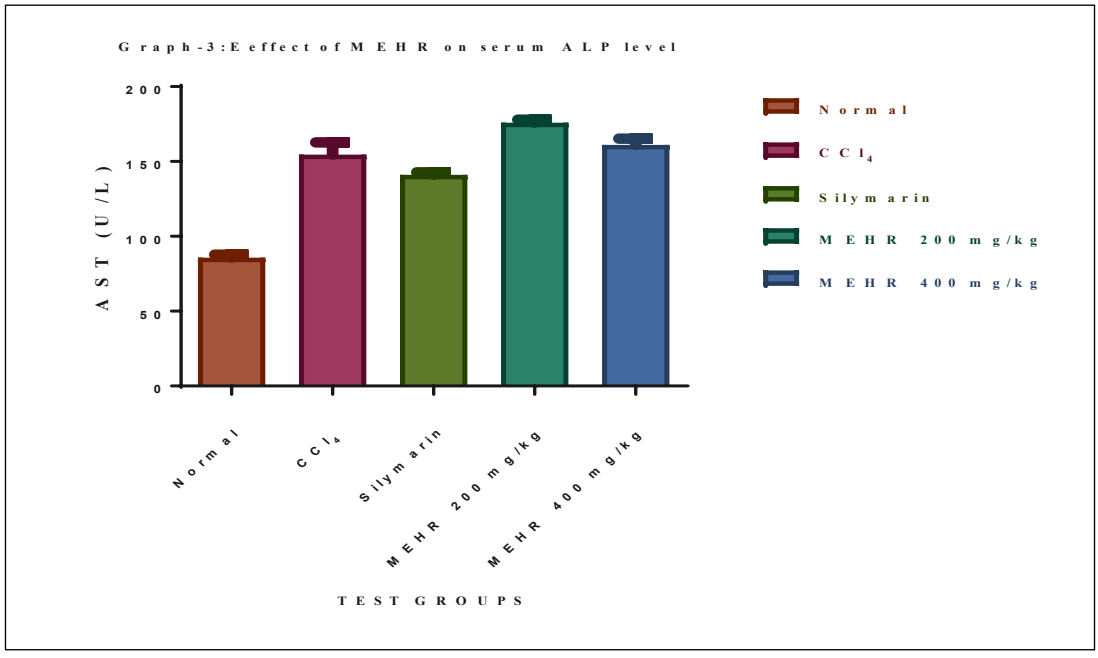


Fig. 5 : Liver sections treated with CCl_4+ MEHR (400 mg/kg, p.o) from Group 5


Graph-1:Effect of MEHR on serum ALT level
ALT (U/L)
250
200
150
100
50
0
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg
TEST GROUPS
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg


Graph-2:Effect of MEHR on serum AST level
AST (U/L)
200
150
100
50
0
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg
TEST GROUPS
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg


Graph-3:Eeffect of MEHR on serum ALP level
AST (U/L)
200
150
100
50
0
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg
TEST GROUPS
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg

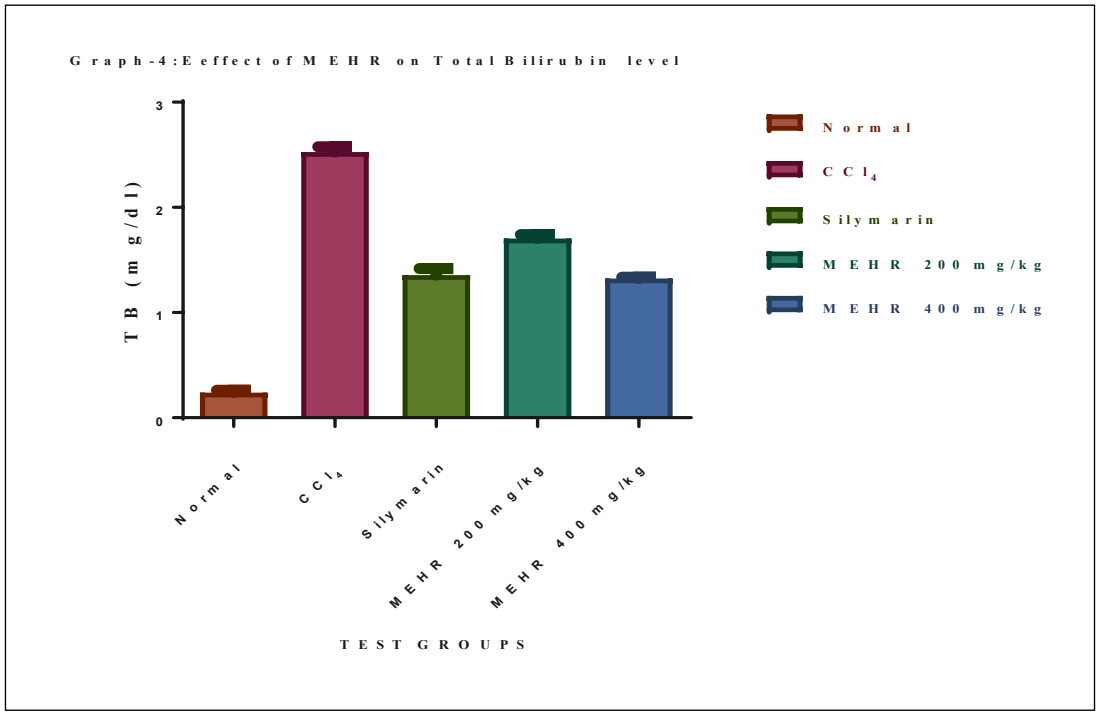

Graph-4:Eeffect of MEHR on Total Bilirubin level
TB (mg/dl)
3
2
1
0
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg
TEST GROUPS
Normal
CCl4
Silymarin
MEHR 200 mg/kg
MEHR 400 mg/kg

Table 2
Effect of MEHR on biochemical parameters in CCl_4 induced hepatotoxicity rats

Groups	Treatment	ALT IU/L	AST IU/L	ALP IU/L	Total bilirubin mg/dl	Total protein g/dl	Albumin g/dl
1	Normal	36.73 ± 12.21	38.00 ± 1.45	86.34 ± 4.37	0.27 ± 0.04	8.44±0.16	4.12 ± 0.14
2	CCl_4 (1mL/kg)	228.14 ± 55.31	142.5 ± 1.33	157.88 ± 6.86	2.58 ± 0.53	5.95 ± 0.05	3.47 ± 1.25
3	Silymarin (100mg/kg)	128.23 ± 21.23***	91.69 ± 1.6***	142.60 ± 1.16 ***	1.32 ± 5.03***	7.44 ± 0.12 ***	3.86 ± 0.12 ***
4	MEHR (200mg/kg)	172.23 ± 38.25***	132.42 ± 16.92	178.15 ± 11.01**	1.69 ± 4.08	6.54 ± 0.24	3.52 ± 0.16 *
5	MEHR (400mg/kg)	161.13 ± 36.22 ***	119.56 ± 17.1 **	161.79 ± 1.51***	1.33 ± 3.07*	7.12 ± 0.69 ***	4.73 ± 0.12 **

n = 6, Data expressed as Mean ± SEM, *P value < 0.05, **P value < 0.01, *** P value < 0.001 compared with toxic group.

DISCUSSION

Histopathological observation of rats administered CCl_4 showed severe necrosis and disappearance of nuclei. This could be due to the rapid formation of highly reactive metabolites, because of administration of CCl_4. All these histopathological changes were significantly reduced in rats treated with methanolic extract of *Hibiscus radiatus* leaves. The study of serum markers such as ALT, AST, ALP, bilirubin, and total protein has been found to be of great value of assess to clinical and experimental liver damage [35]. In the present investigation, the rats suffered significant hepatic damage from treatment with CCl_4, as indicated by elevated levels of serum markers (Table 2). A rise in AST is usually accompanied by an increase in ALT, which plays a vital role in the conversion of amino acids to keto acids [36]. Pretreatment with methanolic extract of *Hibiscus radiatus* leaves, both at 200 mg/kg body weight and 400 mg/kg body weight, significantly attenuated elevated levels of serum markers. This suggests that methanolic extract of *Hibiscus radiatus* leavesconditions the hepatocytes so as to protect the integrity of the membrane from CCL_4-induced leakage of serum markers into circulation. These changes can be considered a functional improvement of hepatocytes and may be caused by accelerated regeneration of parenchyma cells. Bilirubin and Serum ALP are related to hepatic cell damage [37]. Increase in serum ALP is due to increased synthesis in the presence of increasing biliary pressure [38]. The decrease in the levels of ALP and bilirubin may be due to the presence of flavonoids and their antioxidant effects which may protect the hepatic cell damage induced by CCL_4. A potential of hepatoprotective property underlying *Hibiscus radiatus*may be attributed to the antioxidative constituents. The plants most commonly used to treat liver disorders are Glycyrrhizaglabra (licorice), Curcuma longa (turmeric) and Camellia sinensis (green tea), and they are all reported to be hepatoprotective due to the powerful anti-oxidative properties [39-42].The presence of flavonoids and tannins in methanolic extract of *Hibiscus radiatus*leaves was confirmed by phytochemical analysis, and these compounds are reported to have antioxidant properties [43].

Conclusion

In conclusion, this is the first study to demonstrate that hepatoprotective activity of *Hibiscus radiatus* leaves against CCl_4-induced hepatotoxicity in rats. The results indicate that MEHR not only enhances hepatic antioxidant enzyme activities and inhibits lipid peroxidation but also suppresses inflammatory responses in CCl_4-induced liver damage. The possible hepatoprotective mechanism correlates with the inhibition of lipid peroxidation through increasing the activities of antioxidant enzymes and the regulation of proinflammatory mediators to maintain the integrity of hepatic cells. It can be potentially developed into a functional food or even a pharmacological agent for the prevention of liver diseases.

Acknowledgments

The authors are grateful to the Nishka scientific and reference laboratory, Uppal, Hyderabad, India, for providing the necessary laboratory facilities and we are also thankful the Management Anwarul Uloom college of Pharmacy, New Mallepally, Hyderabad (affiliated to Jawaharlal Nehru Technological University- Hyderabad) for their support and facilities.

References

1. Wolf PL. Biochemical diagnosis of liver disease. Indian J ClinBiochem 1999; 14(1):59–90.
2. Piñeiro-Carrero VM, Piñeiro EO. Liver. Pediatrics 2004; 113(4):1097–106.
3. Lee SH, Heo SI, Li L, Lee MJ, Wang MH. Antioxidant and hepatoprotective activities of CirsiumsetidensNakai against CCl4-induced liver damage. Am J Chin Med 2008; 36(1):107–14.
4. Kiso Y, Tohkin M, Hikino H, Hattori M, Sakamoto T, Namba T. Mechanism of antihepatotoxic activity of glycyrrhizin. I: Effect on free radical generation and lipid peroxidation. Planta Med 1984; 50(4):298–302.
5. Ayaz M, Junaid M, Ahmed J, Ullah F, Sadiq A, Ahmad S, Imran M. Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from Polygonumhydropiper L. BMC Complement Altern Med 2014; 14:145.
6. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry. 2001; 56:237–43.
7. Pradeep K, Mohan CV, Gobianand K, Karthikeyan S. Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. Eur J Pharmacol 2007; 560:110–6.
8. Rainone F. Milk thistle. Am Fam Physician 2005;72:1285–8.
9. Lucena MI, Andrade RJ, de la Cruz JP, Rodriguez-Mendizabal M, Blanco E, Sánchez de la Cuesta F. Effects of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo-controlled clinical study. Int J ClinPharmacolTher 2002; 40:2–8.
10. Montvale NJ. Milk thistle (*Silybummarianum*) in PDR for Herbal Medicines. Med Econom Comp. Lewiston: 4th Edition. Thomson Healthcare, 2000:516–20.
11. Kren V, Walterová D. Silybin and silymarin—new effects and applications. Biomed Pap Med FacUnivPalacký Olomouc Czech Repub. 2005; 149:29–41.

12. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. Clin Drug Investig. 2002; 22:51–65.

13. Buzzelli G, Moscarella S, Giusti A, Duchini A, Marena C, Lampertico M. A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB1016) in chronic active hepatitis. Int J ClinPharmacolTherToxicol. 1993; 31:456–60.

14. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E. Pharmacokinetic studies on IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. Eur J Drug MetabPharmacokinet. 1990; 15:333–8.

15. Pareek A, Godavarthi A, Issarani R, Nagori BP. Antioxidant and hepatoprotective activity of *Fagoniaschweinfurthii* (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. J Ethnopharmacol. 2013; 150:973–81.

16. Krithika R, Mohankumar R, Verma RJ, Shrivastav PS, Mohamad IL, Gunasekaran P, Narasimhan S. Isolation, characterization and antioxidative effect of phyllanthin against CCl4-induced toxicity in HepG2 cell line. ChemBiol Interact. 2009; 181:351–8.

17. Harries HM, Fletcher ST, Duggan CM, Baker VA. The use of genomics technology to investigate gene expression changes in cultured human liver cells. ToxicolIn Vitro. 2001; 15:399–405.

18. Bajt ML, Knight TR, Lemasters JJ, Jaeschke H. Acetaminophen-induced oxidant stress and cell injury in cultured mouse hepatocytes: protection by N-acetyl cysteine. Toxicol Sci. 2004; 80:343–9.

19. Rivera CA, Bradford BU, Hunt KJ, Adachi Y, Schrum LW, Koop DR, Burchardt ER, Rippe RA, Thurman RG. Attenuation of CCl_4-induced hepatic fibrosis by GdCl3 treatment or dietary glycine. Am J PhysiolGastrointest Liver Physiol. 2001; 281:G200–7.

20. Ingawale DK, Mandlik SK, Naik SR. Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): a critical discussion. Environ ToxicolPharmacol. 2014; 37:118–33.

21. Pattern CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guergerich FP, Yang CS. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem Res Toxicol. 1993 ;6:511–8.

22. Nelson SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin Liver Dis. 1990; 10:267–78.

23. Holownia A, Braszko JJ. Acetaminophen alters microsomal ryanodine Ca^{2+} channel in HepG2 cells overexpressing CYP2E1. BiochemPharmacol. 2004; 68:513–21.

24. Wang ML, Morris B, Tonnis B, Davis J, Pederson GA. Assessment of oil content and fatty acid composition variability in two economically important *Hibiscus* species. Journal of Agricultural and Food Chemistry 2012; 60(26):6620–6626.

25. Lawton BP. Hibiscus: Hardy and Tropical Plants for the Garden. Edn 1, Timber Press, Portland2004:122-24.

26. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Netherlands, Springer 2012:78.

27. Kokate CK. Practical Pharmacognosy, Delhi, VallabhPrakashan, 1997:107-11.

28. Khandelwal KR. Practical Pharmacognosy, Pune, NiraliPrakashan, 2004:149-60.

29. Anonymous. OECD guidelines for the testing of Chemicals, Acute oral toxicity up and down procedure 2006; 1-8.

30. Janbaz KH, Gilani AH. Studies on preventive and curative effects of berberine on chemical-induced hepatotoxicity in rodents. Fitoterapia, 2000; 71:25-33.

31. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. American Journal of Clinical Pathology, 1957, 28: 53–56.

32. King J. The hydrolases-acid and alkaline phosphatases, Practical Clinical Enzymology, Nostrand Company Limited, London, 1965; 191–208.

33. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colourimeter. Journal of Biological Chemistry 1937; 119:481–490.

34. Prasanna H, Hukkeri VI, Hunasagi BS, Mirihal SC, Kulkarni RV, Patel CC. Screening of Hedychiumspicatum for hepatoprotective activity in albino rats. Indian Drugs 2002; 39 (2):117-119.

35. Moore M, Thor H, Moore G, Nelson S, Moldeus P, Correnius S: The toxicity of acetaminophen and N-acetyl P-benzoquinone imine in isolated hepatocytes is associated with thio depletion and increased cytosolic Ca2+. J BiolChem 1985, 260:13035–13040.

36. Vaishwanar I, Kowale CN: Effect of two ayurvedic drugs Shilajeet and Eclinol on changes in liver and serum lipids produced by carbon tetrachloride. Ind J ExpBiol 1976, 14:58–61.

37. Dash Deepak K, YeligarVeerendra C, Nayak Siva S, TirthaGhosh, Rajalingam D, PinakiSengupta, MaitiBhim C, MaityTapan K: Evaluation of hepatoprotective and antioxidant activity of Ichnocarpusfrutescens (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Trop J Pharm Res 2007, 6(3):755–765.

38. Sallie R, Tredger JM, William: Drugs and the liver. Biopharm Drug Dispos 1999, 12:251–259.

39. Donatus IA, Sardjoko, Vermeulen NP: Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. BiochemPharmacol 1990, 39:1869–1875.

40. Soni KB, Lahiri M, Chackradeo P: Protective effect of food addities on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer Lett 1992, 115:115–121.

41. Wang GS, Han ZW: The protective action of Glycyrrhiza flavonoids against carbon tetrachloride hepatotoxicity in mice. Yao HsuehHsuehPao 1993, 28:572–576.

42. Miyagawa C, Wu C, Kennedy DO: Protective effect of green tea extract and tea polyphenols against cytotoxicity of 1,4-naphthoquinone in isolated rat hepatocytes. BiosciBiotechnolBiochem 1997, 61:1901–1905.

43. Shankar MB, Parikh JR, Geetha M, Mehta RS, Saluja AK: Hepatoprotective activity of benzopyrone from Tephrosiapurpurea Pers. J Nat Rem 2005, 5(2):115–120.

Science – Tech Foundation

Journal of Pharmacy and Chemistry

(An International Research Journal of Pharmaceutical and Chemical Sciences)
(Indexing in CAS)

Plot No. 22, Vidyut Nagar, Anantapur – 515 001(A.P), INDIA

MEMBERSHIP FORM

Dear Editor,

I/We wish to be Annual Member / Life Member of Journal of Pharmacy and Chemistry (An International Research Journal of Pharmaceutical and Chemical Sciences) and agree to abide by your rules and regulations

1. Name in full: _____ Male ☐ Female ☐
   (In block letters)

2. Nationality: _____ 3. Date of birth:_____

3. Corresponding Address: _____

   _____

   _____

5. Institutional / Business Address: _____

6. Phone : (O): _____ (R): _____ E-mail :_____

7. Academic Qualifications : _____

8. Profession and Experience : _____

9. Present Position / Post : _____

10. Pharmaceutical / Chemical interest: _____

Membership Fees	Life	Annual
Individual	Rs. 8000/- US $ 800	Rs. 2000/- US $ 150
Institutional	Rs. 20,000/- US $ 2000	Rs. 2500/- US $ 200

Rs. / $ _____ remitted by Bank / Cash

DD No. _____ Date _____ Banker's Name_____

DD's are drawn in favour of Science-Tech Foundation, Payable at Anantapur.

Place: _____

Date: _____

(Signature of the applicant)

Note: (i) All authors should be member of this Journal, (ii) Saperate Photostat copy may be filled and send.

INSTRUCTION TO AUTHORS

GENERAL REQUIREMENTS: Journal of Pharmacy and Chemistry (ISSN 0973-9874) is a quarterly Journal, ***Indexing in CAS(Coden:JPCOCM)*** which publishes original research work that contributes significantly to further the scientific knowledge in Pharmaceutical Sciences (Pharmaceutical Technology, Pharmaceutics, Biopharmaceutics, Pharmacokinetics, Pharmaceutical Chemistry, Computational Chemistry and Molecular Drug Design, Pharmacognosy and Phytochemistry, Pharmacology, Pharmaceutical Analysis, Pharmacy Practice, Clinical and Hospital Pharmacy, Cell Biology, Genomics and Proteomics, Pharmacogenomics, Stem Cell Research, Vaccines & Cera, Bioinformatics and Biotechnology of Pharmaceutical Interest) and in Chemical Sciences (Inorganic, Soil, Forensic, Analytical, Nano, Environmental, Polymer, Physical, Agricultural, Medicinal, Biochemistry, Organic, Computational, Food, Pesticides etc). Manuscripts are accepted for consideration by Journal of Pharmacy and Chemistry on the condition that they represent original material, have not been published previously, are not being considered for publication elsewhere, and have been approved by each author. Review articles, research papers, short communication and letters to the editor may be submitted for publication.

SUBMISSION OF MANUSCRIPTS: Typewritten manuscripts prepared using MS Word should be submitted in triplicate and RW-CD to Prof. Dr. K.N Jayaveera, Editor-in-Chief of Journal of Pharmacy and Chemistry , Plot No 22, Vidyut Nagar, Ananthapur- 515 001, Andhra Pradesh, India. e-mail: editorjpc@gmail.com

All components of the manuscript must appear within a single electronic file: references, figure legends and tables must appear in the body of the manuscript.

TYPING INSTRUCTION: The following detailed instructions are necessary to allow direct reproduction of the manuscript for rapid publishing. If instructions are not followed, the manuscript will be returned for retyping. The following typefaces, in 12 points size, are preferred: Times Roman.

GENERAL FORMAT: The typing area must be exactly 6 5/8" (168 mm) wide by 9 7/8" (250 mm) long. Justify margins left and right (block format). The entire typing area of each page must be filled, leaving no wasted space. Text should be double-spaced, special care should be taken to insure that symbols, superscripts and subscripts are legible and do not overlap onto lines above or below. Make sure text lines are equidistant.

TITLE: On the first page of the manuscript, start title 1" (25 mm) down from top text margin. Type title in all capital letters, centred on the width of the typing area and single-spaced if more than one line is required. The title should be brief, descriptive and have all words spelled out. Double-space, then type the author(s) name(s), single-spaced if more than one line is required. Double-space, than type author(s) address(es), single-spaced, capitalizing first letters of main words. Quadruple-space before Abstract.

ABSTRACT: Centre, type and underline abstract heading, capitalizing the first letter. A double-space should separate the heading from the abstract text. Indent abstract text approximately 1/2" (13 mm) from both left and right margins. The abstract should be intelligible to the reader without reference to the body of the paper and be suitable for reproduction by abstracting services. Introduction to the text (without a heading) should being four spaces below the abstract using full margins.

KEY WORDS: Three or more key words must be provided by authors for indexing of their article. Key words will be listed directly below the Abstract. Abbreviated forms of chemical compounds are not acceptable. Spell out entirely, using the official nomenclature. Example: L-dihydroxyphenylalanine (L-DOPA)

MAJOR HEADINGS: Papers must include the major headings: Introduction, Methods, Results, Discussion, Acknowledgments and References. Capitalize first letter, underline, and centre headings on width of typing area.

TABLES/FIGURES: Incorporate tables and/or figures (B & W) with their legends into the main body of text.

REFERENCES: Referneces should be referred to a number [1] in the text and be listed according to this numbering at the end of the paper. Only papers and books that have been published or in press may be cited; unpublished manuscripts or manuscripts submitted to a journal but which have not been accepted may not be cited.

The references shoulld comprise the following information and in the given order and with given punctuation as given in the example below: Author name (s), Initials (s), Publication Title, Page Number, Year of Publication.

Standard Journal Article:

[1] Bhattacharyya D, Pandit S, Mukherjee R, Das N, Sur TK. Indian J Physiol Pharmacol 2003; 47:435.

[2] Skottova N, Krecman V. Physiol Res 1998; 47:1.

Book:

[1] Ghosh MN. Fundamentals of Experimental Pharmacology, 2nd ed. Calcutta Scientific Book Agency, 1984:154.

Proofs will be sent to the corresponding author. These should be returned as quickly as possible.

The facts and view in the article will be of the authors and they will be totally responsible for authenticity, validity and originality etc. the authors should give an undertaking while submitting the paper that the manuscripts submitted to journal have not been published and have not been simultaneously submitted or published elsewhere and manuscripts are their original work.

www.jpc.stfindia.com
www.stfindia.com

This Quarterly Journal is Printed, Published, Owned and Edited by Dr. K.N. Jayaveera, Published from Science-Tech Foundation, Plot No.22, Door No. 12/3/925, Vidyut Nagar, Anantapur - 515 001. Send your queries at www.stfindia.com and Printed at Vipla Computer Services (Designers & Multi Colour Offset Printers) # 2-3-756/2, Golnaka, Amberpet, Hyderabad - 500 013.