IJBPAS, January, 2013, 2(1): 80-89

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

HEPATOPROTECTIVE ACTIVITY OF *Sophora interrupta* AND *Holoptelea integrefolia* AGAINST CARBONTETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

HEMAMALINI K* AND SATHYA SB

Associate Professor, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad

Corresponding Author: E Mail: iictrrcp@gmail.com

ABSTRACT

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals. The objective of this study was to investigate the hepatoprotective activity of methanolic extracts of *Sophora interrupta and Holoptelea integrefolia* against CCl_4 induced hepatotoxicity. Animals were pretreated with the methanolic extracts of *Sophora interrupta* (400 mg/kg) and *Holoptelea integrefolia* (500 mg/kg) for one week and then challenged with CCl_4 (1.5 ml/kg) in olive oil (1:1, v/v) on 7^{th} day. Serum marker enzymes (ALP, AST, ALT and Total bilirubin) were estimated in all the study groups. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and Total bilirubin were tested in both CCl_4 treated and extract treated groups. CCl_4 has enhanced the AST, ALT and ALP in liver. Treatment of methanolic extracts of *Sophora interrupta* (400 mg/kg) and *Holoptelea integrefolia* (500 mg/kg) exhibited a significant protective effect by altering the serum levels of AST, ALT, ALP and Total bilirubin. These biochemical observations were supported by histopathological study of liver sections. From this preliminary study it has been concluded that among the two extracts tested, the methanolic extract of *Sophora interrupta and Holoptelea integrefolia* found to possess significant protective effect against CCl_4 induced hepatotoxicity.

Keywords: *Sophora interrupta, Holoptelea integrefolia,* CCl_4, Hepatoprotective

INTRODUCTION

Liver - a major metabolic organ affected by various chemicals and toxins daily and identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief **[1]**. Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free redicals **[2]**.

Sophora interrupta- Bedd commonly known as "*Pili Girgoli* **[3]**" is a woody perennial shrub which grows endemically in seshachalam hill ranges, seshatheertham and kumaradhara theertham in Tirumala, India, and belonging to the family "*Leguminosae*". It contains Matrine, Oxymatrine type of Alkaloids **[4, 5]**, Flavonoids **[6, 7]**, Saponins and Polysaccharides **[8]**.

It possess wide-reaching pharmacological actions, including anti-oxidant, anti-cancer, anti-asthmatic, anti-neoplastic, antimicrobial, anti-viral, antidote, anti-pyretic, cardio tonic, anti-inflammatory, diuretic and in the treatment of skin diseases like eczema, colitis and psoriasis.

Holoptelea integrifolia (Roxb.) Planch is commonly known as *"Indian Elm"* which belongs to the family "*Ulmaceae"* is a large deciduous tree, gowing up to 18m tall. It contains Carbohydrates, Proteins, Amino acids, Steroids, Glycosides, Alkaloids, Tannins and Phenolics **[9]**. It is reported to have antiviral **[10]**, antioxidant, antimicrobial and wound healing activity **[11]**. Ethno medically, the leaves and stem bark of this plant were used by tribal for skin diseases, obesity **[12]** and in the management of cancer **[13]** .The study was conducted to establish the traditional use of *Sophora interrupta* and *Holoptelea integrefolia* as hepatoprotective against CCl_4 induced hepatotoxicity in rats. In the view of scientific report, the leaves of *Sophora interrupta* and *Holoptelea integrefolia* was evaluated against CCl_4 induced hepatic damage in rats with the aim of developing a natural hepatoprotective drug.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *Sophora interrupta* and *Holoptelea integrefolia* were collected from Sri Venkateshwara University, Tirupati, Andhra Pradesh, India, in June 2010 **[14]**. The plant was authenificated by a Botanist, Dr. K. Madhava Chetty, Assistant professor, Department of Botany and voucher specimen was deposited in Sri Venkateshwara University, and a copy has been preserved for the future reference at the herbarium of the institute TRRCP. After authentification, the leaves were cleaned and shade dried and milled into coarse powder by a mechanical pulverizer.

Preparation of Extract

The coarse powder of plant material was defatted with petroleum ether (60-80°C) in a soxhlet extraction apparatus and marc was extracted with methanol (1000 ml). Overnight, at room temperature with constant stirring.

The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. Extract was dried in dessicator. The crude extract was suspended in 1% Tween-80 to required concentrations and used for the experiments.

Phytochemical Screening

The methanolic extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents **[15]**. The phytoconstituents in the extract was found to contain alkaloid, flavonides, glycosides, steroids and tannins.

Formulation

Suspensions were formulated of required concentrations 300 mg/kg and 500 mg/kg by using 1% Tween-80 and double distilled water. The formulated suspensions were compared for various evaluation parameters.

Pharmacological Studies

Animals

Male Wistar rats weighing between 150-200 gm were used for this study. The animals were obtained from NIN, Hyderabad, India. The animals were placed in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the CPCSEA (No. 1447/PO/a/11/CPCSEA).

Hepatoprotective Activity

Animals were divided into 5 groups of six rats each. Group- I and II served as normal and toxic control, and received only the vehicle (1% Tween-80; 1 ml/kg; p.o). Group- III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group- IV and group- V received the *Sophora interrupta* and *Holoptelea integrefolia* extract at an oral dose of 400 mg/kg and 500 mg/kg respectively, as a fine suspension of 1% Tween-80. The treatment was continued for 7days, once daily. On the day of 7 for groups II - V, 30 min post-dose of extract administration animals received CCl_4 at the dose of 1.5 ml/kg (1:1 v/v of CCl_4 in olive oil) orally **[16, 17, 18]**.

Biochemical Estimation

The animals were sacrificed 36h after administration of acute dose of CCl_4. The blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) levels were estimated from the serum by using standard kits **[19]**.

Histopathological Studies

The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any **[18]**.

RESULTS

The results were shown in the **Table 1**. The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's't' - test. P values <0.01 were considered significant.

Biochemical Parameters

The results of hepatoprotective activity of methanolic extracts of *Sophora interrupta* and *Holoptelea integrefolia* on Carbon tetrachloride treated rats are shown in **Figure 1**. The hepatic enzymes ALT (111.2 ± 1.35), AST (100.5 ± 0.76), ALP (102.3 ± 0.91) and bilirubin (2.11 ± 0.04) in serum was significantly increased in paracetamol treated animals when compared to control. The methanolic extract of Sophora interrupta and Holoptelea integrefolia treatments significantly reversed the levels of ALT (68.50 ± 0.42; 70.67 ± 0.66), AST (70.17 ± 1.85; 66.50 ± 0.76), ALP (43.83 ± 1.07; 44 ± 1.31) and bilirubin (0.46 ± 0.01; 0.45 ± 0.01) when compared to Carbon tetrachloride alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in ALT (63.17 ± 0.79), AST (62.50 ± 0.76),

ALP (40.83 ± 0.60) and bilirubin (0.40 ± 0.003) levels when compared to Carbon tetrachloride alone treated rats.

Histopathological Studies

The histopathological studies are direct evidence of efficacy of drug as protectant. Simultaneous treatment of methanolic extracts of *Sophora interrupta* and *Holoptelea integrefolia* with CCl4 exhibits less damage to the hepatic cells as compared to the rats treated with CCl4 alone. Histological changes such as steatosis (fatty changes in hepatocytes), inflammatory infiltrations and perivenular fibrosis were observed in CCl_4-treated (toxic) control group. Both the extracts prevented these histological changes, further indicating their hepatoprotective activity. All the histological changes observed were in correlation with the biochemical, antioxidant and functional parameters of the liver **(Figure 2 a-e)**.

Table 1: Effect of *Sophora interrupta and Holoptelea integrefolia* on Serum Marker Enzymes (ALT, AST, ALP) and Total Bilirubin on CCl_4 Induced Hepatotoxicity in Rats

GROUPS	ALT(µ/L)	AST(µ/L)	ALP(µ/L)	TB(µ/L)
Normal Control	57± 1.065	52.83 ± 1.5	34 ± 1.183	0.3915± 0.0006
CCl_4 Control	112.2 ± 1.35*	100.5± 0.76*	102.3 ± 0.9189*	2.12 ± 0.04*
METR	68.50 ± o.42$^{\dagger@}$	70.17 ± 1.85$^{\dagger@}$	43.83 ± 1.07$^{\dagger@}$	0.46 ± 0.011$^{\dagger@}$
MESP	70.67 ± 0.66$^{\dagger@}$	66.50 ± 0.76$^{\dagger@}$	44 ± 1.31$^{\dagger@}$	0.45 ± 0.011$^{\dagger@}$
Silymarin	63.17 ± 0.79†	62.50 ± 0.76†	40.83 ± 0.60†	0.40 ± 0.003†

METR, Methanolic Extract of *Sophora interrupta*; MESP, Methanolic Extract of *Holoptelea integrefolia*; Values are Expressed as Mean ± SEM for Six Rats in each Group; *P≤0.01 when Compared to Control; †P≤0.01 when Compared to CCL_4; $^{@}$P≤0.01 when Compared to Silymarin

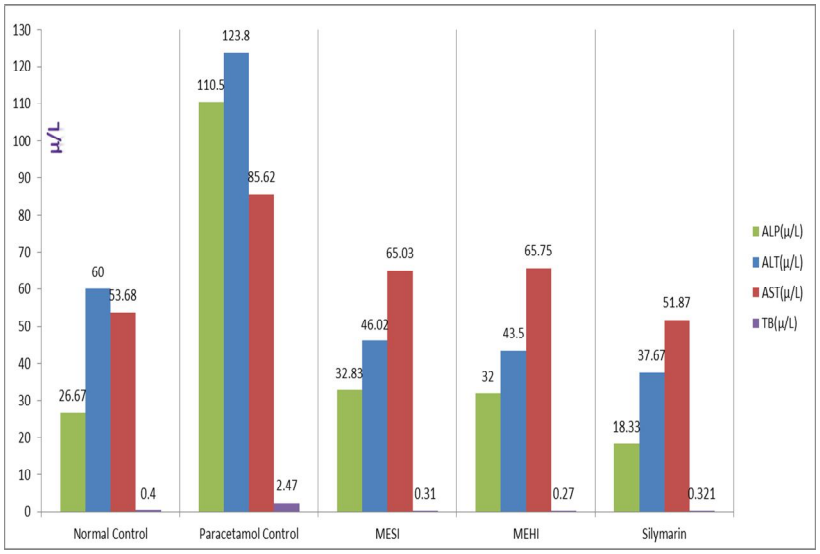

Figure 1: Effect of Methanolic Extract of *Sophora interrupta* and Methanolic Extract of *Holoptelea integrefolia* and Silymarin on Carbon Tetrachloride Induced Hepatotoxicity Model in Rats

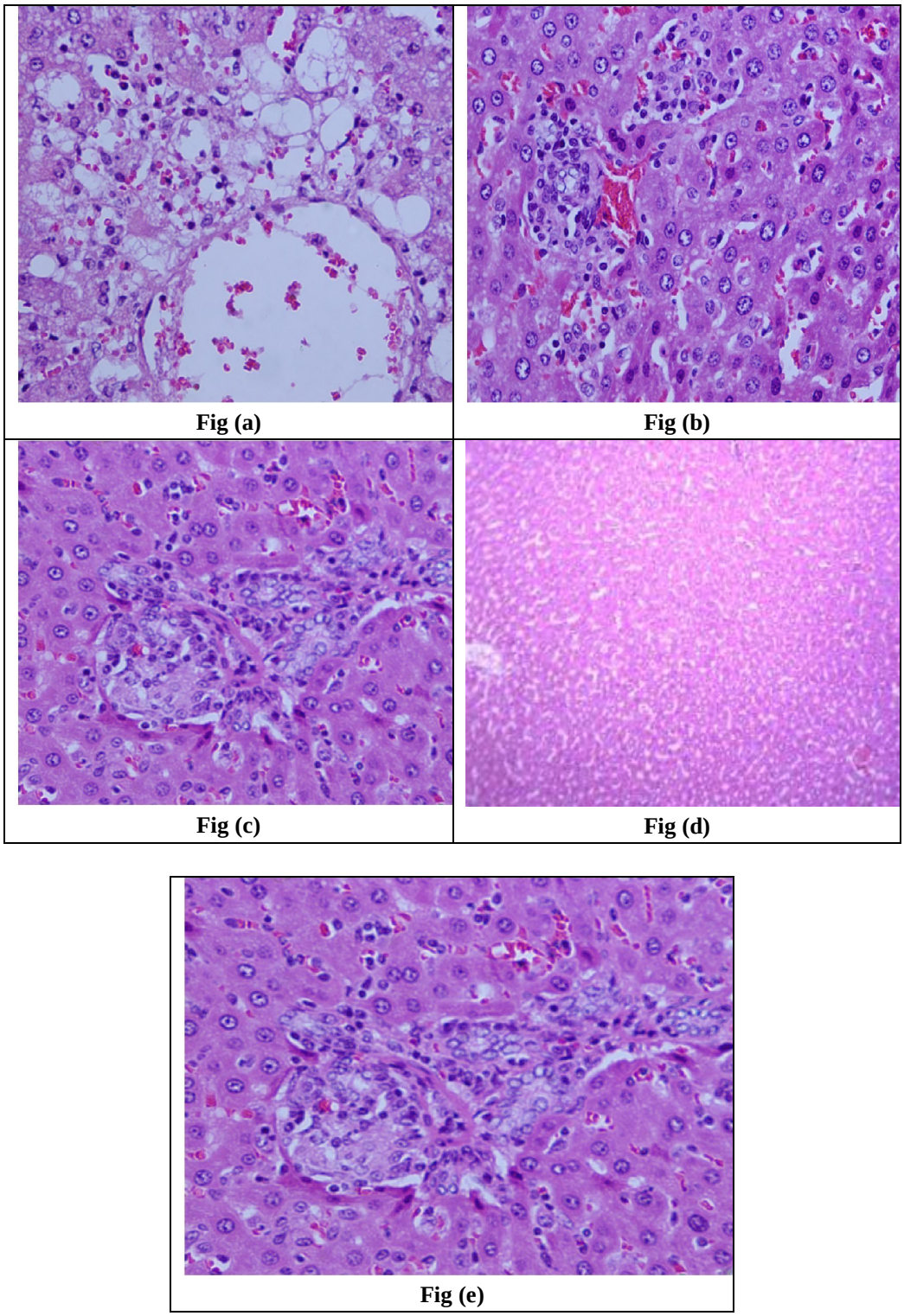

Figure 5: Histology of Liver Sections (a) Section of the Liver Tissue of Animal Treated with CCl_4; (b) Section of Liver Tissue of Methanolic Extract of *Tabebuia rosea* Treated Animal; (c) Section of Liver Tissue of Methanolic Extract of *Solanum pubescens* Treated Animal; (d) Section of the Liver Tissue of Control Animal; (e) Section of the Liver Tissue of Silymarin Treated Animal

DISCUSSION

A number of chemicals including various environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. CCl_4 is one of such extensively studied environmental toxicant [**2]**. Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Toxicity in liver due to CCl_4 and other chemicals is attributed to the toxic metabolites formed, responsible for the initiation of CCl_4 dependent lipid peroxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the trichloromethyl radical **[1]**. In the liver, CCl_4 is metabolized by the cytochrome P450-dependent monooxygenase systems followed by its conversion to more chemically active form, trichloromethyl radical. The enzymes involved in this process are located in the endoplasmic reticulum of the liver and their activities are dependent on many environmental factors. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties **[2]**.

Present study was conducted to evaluate the protective effect of the *Sophora interrupta* and *Holoptelea integrefolia* against CCl_4 induced hepatic damage in rat. Results suggest that the extract possesses protective action against hepatic dysfunction induced by the potent toxin CCl_4. Both biochemical and histopathological data showed that there was no difference in extract treatment when compared with standard drug silymarin. Extensive evidence demonstrated that trichloromethyl radical are formed as a result of the metabolic activation of CCl_4, which in turn, initiate lipid peroxidation process. A known potent antioxidant, vitamin E, could protect CCl_4 induced liver injury indicating that oxidative stress is responsible for CCl_4 induced hepatic disorder in this particular model **[20, 21]**. *Sophora interrupta, Holoptelea integrefolia* and silymarin treated groups significantly protect organ against CCl_4 induced hepatic damage. Our results suggest that the two methanolic extract tested, *Sophora interrupta* and *Holoptelea integrefolia* possesses hepatoprotective activity.

CONCLUSION

The methanolic extract of *Sophora interrupta and Holoptelea integrefolia* extract has shown the ability to maintain the normal functional status of the liver. From the above

preliminary study, we conclude that the methanolic extract of *Sophora interrupta and Holoptelea integrefolia*, is proved to be one of the herbal remedies for liver ailment.

ACKNOWLEDGEMENT

The authors are grateful to the secretary Mr. T. Dinesh Reddy of Teegala Ram Reddy College of Pharmacy, Hyderabad, India for providing the facilities for this work.

REFERENCES

[1] Arulkumaran KSG, Rajasekaran A, Ramasamy R, Jegadeesan M, Kavimani S and Somasundaram A, Cassia roxburghii seeds protect Liver against Toxic effects of Ethanolic and Carbon tetrachloride in rats, Int. J. Pharm. Tech. Res., 1(2), 2007, 273-246.

[2] Sil PC, Manna P and Sinha M, Aqueous extract of Terminalia arjuna prevents carbon tetrachloride induced hepatic and renal disorders, BMC Complementary Alternative Medicine, 6, 33, 2006.

[3] Singh AN, Verma DM, Flora of Madhya Pradesh, 1993

[4] Liu M, Liu XY and Cheng JF, Advance in the pharmacological research on matrine, Zhongguo Zhong Yao Za Zhi, 28, 2003, 801-804.

[5] Zhang YF, Wang SZ, Li YY, Xiao ZY, Hu ZL and Zhang JP, Sophocarpine and matrine inhibit the production of TNF and IL-6 in murine macrophages and prevent cachexia-related symptoms induced by colon 26 adenocarcinoma in mice, Int. Imm. pharmacol., 8, 2008, 1767-1772.

[6] Minhaj N, Khan H and Zeman A, Unanisoflavan, a new isoflavan from *Sophora secondiflora* DC, Tetrahedron Lett., 27, 1976, 2391–2394.

[7] Minhaj N, Khan H and Zaman A, Secondifloran, a novel isoflavanone from *Sophora secondiflora* DC, Tetrahedron Lett., 13, 1977, 1145–1148.

[8] Ohyama M, Tanaka T and Iinuma M, Two novel resveratrol trimers, Leachianols A and B, from *Sophora leachiana*, Chem. Pharm. Bull., 42, 1994, 2117-212.

[9] Dalsaniya NO, Patel DV, Patel HV, Bharat RG, Kaji BC and Bhagat GR, Allergen skin test, Analysis of results in patients of bronchial asthama, Holoptelia integrefolia, J. Ind. Practitioner, 52 (6), 1999, 402-406.

[10] Rajbhandari M, Wegner U, Jülich M, Schopke T and Mentel R, Screening

of Nepalese medicinal plants for antiviral activity, J. Eth. Pharmacol., 74, 2001, 251-5.

[11] Srinivas RB, Kiran K, Reddy R, Naidu VG, Madhusudhana K, Agwane SB and Ramakrishna S, Evaluation of antimicrobial, antioxidant and wound-healing potentials of *Holoptelea integrifolia*, J. Ethnopharmacol., 2008, 115, 2008, 249-56.

[12] Bambhole VB and Jiddewar GG, Anti-obesity effect of *Iris versicolor* and *Holoptelea integrifolia* in rats, Sachitra Ayurved., 37, 1985, 557-61.

[13] Graham JG, Quinn ML, Fabricant DS and Farnsworth NR, Plants used against cancer-an extension of the work of Jonathan Hartwell, J. Eth. Pharmacol., 73, 2000, 347-77.

[14] Madhava CK, Sivaji K and Tulasi RK, Flowering plants of Chittor District, Andhra Pradesh, India, 2008, 330-333.

[15] Harbone JB, and Baxter HH, Phytochemical Dictionary: A hand Book of Bioactive Compound from plants, Taylor and Francis, Washington, D.C., U.S.A, 1993, 237.

[16] Saraf S and Dixit VK, Hepatoprotective activity of Tridax procumbens part- 2, Fitoterapia, 62, 1991, 534-536.

[17] Mohideen S, Ilavarasan R, Sasikala E and Kumarn TR, Hepatoprotective activity of *Nigella sativa* Linn, Ind. J. Pharm. Sci., 2003, 65:550-551.

[18] Gnanaprakash K, Madhusudhana CC, Ramkanth S, Alagusundaram M, Tiruvengadarajan VS, Angala Parameswari S and Mohamed Saleem TS, Aqueous Extract of Flacourtia indica Prevents Carbon Tetrachloride Induced Hepatotoxicity in Rat, Int. J. Biolog. and Life Sci., 6, 1, 2010,

[19] Rajesh KG, Achyut NK, Geeta W, Murthy PS, Ramesh C and Vibha T, Nutritional and Hypoglycemic Effect of Fruit Pulp of Annona squamosa in Normal Healthy and Alloxan-Induced Diabetic Rabbits, Ann. Nutr. Metab., 49, 2005, 407-413.

[20] Yoshikawa T, Furukawa Y, Murakami M, Takemura S and Kondo M, Effect of vitamin E on D-Galactosamine-induced or carbon tetrachloride-induced hepatotoxicity, Digestion, 25, 1982, 222-229.

[21] Webwr LW, Boll M and Stampfl A, Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, Crit. Rev. Toxicol., 33, 2003, 105-136.