

## **REPORT**

# **Comparative phytochemical, hepatoprotective and antioxidant activities of various samples of *Swertia Chirayita* collected from various cities of Pakistan**

**Sidra Mahmood\*<sup>1</sup>, Shahzad Hussain<sup>2</sup>, Sobia Tabassum<sup>1</sup>, Farnaz Malik<sup>2</sup> and Humayun Riaz<sup>3</sup>**

<sup>1</sup>Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan

<sup>2</sup>National Institute of Health, Islamabad, Pakistan

<sup>3</sup>Department of Pharmacy, Sargodha University, Sargodha, Pakistan

**Abstract:** Medicinal plants are crucial for about 80% of the world population in developing and developed countries for their primary and basic health care needs owing to better tolerability, superior compatibility with human body and having lesser side effects. The present study was conducted on various solvent extracts of three plant samples of Indian and Nepali origin *Swertia Chirayita* (Roxb.) Buch-ham (Chiratia) collected from various places to establish their comparative phytochemical analysis, chromatographic profile, hepatoprotective and antioxidant activities. Nepali *Swertia Chirayita* was found to have finest Chromatographic profile (TLC). Phytochemical analysis revealed Alkaloids, flavonoids, saponins, ascorbic acid, glycosides, steroids and triterpenoids in all samples. Different solvent fractions of the methanolic plant extracts of *Swertia chirayita* were assessed for hepatoprotective activity by carbon tetrachloride-induced liver damage in rats. The grade of protection was measured by using biochemical parameters such as serum glutamate oxalate transaminase (SGOT/AST), alkaline phosphatase (ALP), serum glutamate pyruvate transaminase (SGPT/ALT) and total bilirubin. The in-vitro antioxidant activity of the extracts was also evaluated by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The methanolic and aqueous extracts, at a dose of 200mg/kg and 300mg/kg, produced significant ( $P < 0.05$ ) hepatoprotection by decreasing the activities of the serum enzymes and bilirubin while there were marked scavenging of the DPPH free radicals by the fractions. Decreased observed in the biochemical parameters suggests that the plant extracts possesses hepatoprotective as well as antioxidant activities without any significant variation amongst them. These activities reside mainly in the methanolic extract of whole plant.

**Keywords:** *Swertia chirayita*, Hepatoprotective, Carbon tetrachloride, mice, antioxidant, phytochemical analysis, DPPH model, Chromatographic profile.

## **INTRODUCTION**

Medicinal plants are an essential stay of about 80% of the world population in developing and developed countries for primary and basic health care needs owing to better cultural tolerability, superior compatibility with human body and having lesser side effects (Hussain *et al.*, 2012). Liver sickness is the chief cause of morbidity and mortality among people, distressing humans of all age groups. WHO assessed that around 170 million people are contaminated with hepatitis C alone and every year 3-4 millions are newly added into the list. In addition, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting contaminated with acute HBV yearly (WHO, 2000). Liver plays a remarkable variety of functions in the upholding, performance and regulating homeostasis of the body and is engaged with approximately all the biochemical alleys to growth, struggle against malady, energy provision, nutrient provision and reproduction (Kristina *et al.*, 2012).

Liver problems are primarily caused by lethal chemicals, surplus utilization of alcohol and autoimmune anarchies. There are also drug induced hepatotoxicities such as liver injury by rifampicin, paracetamol, isoniazid, acetaminophen and chloroquine (Zimmerman, 1978; Tasduq *et al.*, 2005) and chemically induced hepatotoxicity i.e. CCl<sub>4</sub> induced hepatotoxicity. Drug-induced liver injury accounts for more than 50% of acute and chronic liver malfunction, which comprises about 39% hepatotoxicity caused by overdose of acetaminophen 13% idiosyncratic liver injury prompted by other drugs (Kaplowitz, 2001; Michael and Cynthia, 2006). The majority of the hepatotoxic chemicals harm hepatic cells, chiefly by inducing lipid peroxidation and other oxidative injuries (Shanmugasundaram and Venkataraman, 2006). Carbon tetrachloride (CCl<sub>4</sub>) has been used widely to study hepatotoxicity in animal models by instigating lipid peroxidation, in that way causing damages to kidney, heart, testis and brain in addition to liver pathogenesis (Khan *et al.*, 2010; Murugesan *et al.*, 2009). Herbal medicines are considered as comparatively harmless and have been used in the management of liver maladies for a

\*Corresponding author: e-mail: sidrah\_mahmood@yahoo.com

long time. The effectiveness of herbal medicines needs to be appraised through carefully designed multicenter clinical studies (Alvari *et al.*, 2012).

*Swertia chirayita* (Roxb. ex Fleming) H. Karst commonly recognized as “East Indian Balmony”, is a tenuous, upright herb belongs to the family Gentianaceae. *S. Chirayita* has been used as herbal medicine in treating a wide variety of diseases in Sub-continent. It is known for its antispasmodic, antioxidant, anti-inflammatory, anti-diabetic, antitussive, antipyretic and antibacterial activities (Saha and Das, 2001; Alam *et al.*, 2007).

In order to examine the effectiveness of hepatoprotective and antioxidant substances, we have employed several methods of free radical scavenging such as reduced glutathione and S-transferase, lipid peroxidation, glutathione peroxidase, catalase peroxidation, superoxide dismutase or *in vitro* techniques such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging models. Hepatic damage is usually done using such drugs as acetaminophen, isoniazid and rifampicin or chemicals such as carbon tetrachloride. Keeping in view the medicinal importance of *S. Chirayita*, the present study has been constructed to evaluate the hepatoprotective and antioxidant activity of *S. Chirayita* collected from various places against liver enzymes in CCl<sub>4</sub> induced BALB/c mice. The initial phytochemical analysis of the extract was also carried out in addition to chromatographic profile.

## MATERIALS AND METHODS

### *Plant materials and preparation of extracts and fractions*

Fresh plant of *S. Chirayita* (Indian and Nepali origin) were collected and their botanical characteristics were resolved and authenticated by taxonomist from NARC (National Agriculture Research Council), Islamabad and voucher specimens were deposited to the Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan in its herbarium with the numbers DCTMD-1051 to 1053-DCTMD respectively.

Plant samples were named as Nepali chirayita Multan, Nepali Chirayita Karachi and Indian Chirayita Multan i.e NCM, NCK and ICM respectively. The plants were washed with water, cut into pieces and air-dried at room temperature. The dried plants were then crushed into coarse powder in a crushing machine. 1 kg of air-dried ground plants were extracted with 2 liters of methanol using soxhlet apparatus. Solvent from the samples was filtered and evaporated off under reduced pressure in a rotary evaporator to obtain crude methanol extract (ME). Then they were transferred into air-tight bottles and separated sequentially with solvents in increasing order of polarity starting with n-hexane, chloroform, ethyl acetate,

methanol to ethanol. Their corresponding soluble fractions were labeled number wise.

### *Animals*

Healthy 6-8 weeks old balb/c mice (20-35g) of each sex were obtained from Animal House of National Institute of Health, Islamabad and were accommodated in standard poly-propylene cages at a 12-h cycle of light and dark. Room temperature was maintained and kept at 22±5°C and 50% humidity. Animals were kept on a standard laboratory diet. Food and water were given ad libitum. All the chemicals used in this experiment were of the systematic category.

### *Experimental design for hepatoprotective activity*

The hepatoprotective activity of the extracts of the plant was tested using carbon tetrachloride (CCl<sub>4</sub>) model. Silymarin (100 mg/kg b.w.) was used as a standard drug in this study. Silymarin is a flavonolignan that has been introduced as a hepatoprotective agent. It is extracted from the seeds and fruits of *Silybum marianum*. It is one of such polyherbal formulation, a complex mixture of four flavonolignan isomers, namely silybin (being primary constituent), isosilybin, silydianin and silychristin (Saller *et al.*, 2001). The mice were divided randomly into nine groups according to the following protocol.

### *Treatment protocol*

Group I: (Normal control) received only normal food and water.

Group II: (Hepatotoxic induction control) received intraperitoneally, 25% CCl<sub>4</sub> dissolved in olive oil at dose of (1 ml/kg of b.w.) once in a week.

Group III: (Drug or positive control) received CCl<sub>4</sub> intraperitoneally once in a week and standard drug silymarin (100mg/kg b.w.) on remaining days of week for seven days.

Group IV-IX: were served as Herb treated groups and further divided into two subgroups group A and Group B.

Group A and B were administered orally aqueous and methanolic extracts of 200mg/Kg and 300mg/Kg of *Swertia Chirayita*. Groups IVA and IVB received CCl<sub>4</sub> intraperitoneally twice a week and aqueous extracts of NCM (200mg/kg b.w. and 300mg/kg b.w.) for remaining seven days. Groups VA and VB received CCl<sub>4</sub> intraperitoneally once a week and methanolic extracts of NCM (200mg/kg b.w. and 300mg/kg b.w.) on remaining days for seven days.

Groups VIA and VIB received CCl<sub>4</sub> intraperitoneally once a week and aqueous extracts of NCK (200mg/kg b.w and 300mg/kg b.w.) on remaining days for seven days.

Groups VIIA and VIIB received CCl<sub>4</sub> intraperitoneally once a week and methanolic extracts of NCK (200mg/kg b.w. and 300mg/kg b.w.) on remaining days for seven days.

Groups VIIIA and VIIIB received CCl<sub>4</sub> intraperitoneally twice a week and aqueous extracts of ICM (200mg/kg b.w. and 300mg/kg b.w.) on remaining days for seven days.

Groups IXA and IXB were received CCl<sub>4</sub> intraperitoneally once a week and aqueous extracts of ICM (200mg/kg b.w. and 300mg/kg b.w.) on remaining days for seven days.

#### Assessment of hepatoprotective activity

The liver protective action was assessed biochemically and histopathologically. The animals were operated under ether anesthesia after 72 hours of drug treatment. Blood from each mouse was taken from carotid artery located at the neck and gathered in already labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was secluded by centrifugation at 3000 rpm for 15 minutes. The isolated serums were utilized for the evaluations of biochemical parameters like ALT, AST, ALP and Bilirubin. Liver tissues were quickly excised and a part of liver was stored in 10% formalin for proper fixation and were procedure and entrenched in paraffin wax. Fragments of 5-6µm in width were cut and blemished with eosin and haematoxylin. Each liver was microscopically seen for distribution of lesions. The outline of liver damage and lesion grading as minimal moderate and maximal were resolute in slides. Liver segments were also noticed for any degenerative changes (Ahsan *et al.*, 2009).

#### Antioxidant activity

Antioxidant property of plant samples were carried out according to the method described by Wojdyło *et al.*, 2005. Samples (1g) were added into 10 ml of 80% aqueous methanol and the suspensions were stirred slightly. Tubes were vortexed twice for 15min and left at room temperature (20°C) for 24 h. The extracts were centrifuged for 10min at 1500 rpm, and supernatants were collected at 4°C prior to use within 24 h.

#### Reducing power Assay

The reducing power of all three plant extracts were determined according to the method reported (Lim and Murtijaya, 2007). Different amounts of the extracts were suspended in distilled water and mixed with 2.5ml of 0.2 M phosphate buffer (pH 6.6) and 2.5ml of 1% K<sub>3</sub>Fe (CN). The mixture was incubated at 50°C for 20 mins. and 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 mins. Whereas 2.5ml of upper layer of the solution was mixed with 2.5ml distilled water and 0.5ml of 0.1% FeCl<sub>3</sub> and the absorbance was measured at 700nm. Increase in absorbance of reaction mixture indicated reducing power.

#### Scavenging of H<sub>2</sub>O<sub>2</sub>

Scavenging of H<sub>2</sub>O<sub>2</sub> was determined by the method reported by Alam *et al.*, 2001. About 4 mM H<sub>2</sub>O<sub>2</sub> 0.6 ml solution (prepared in PBS) was added to 4 ml solution of

extract and incubated for 10 min. The absorbance of the solution was measured at 230 nm by using spectrophotometer method. Free radical scavenging potency as determined from % age H<sub>2</sub>O<sub>2</sub>. Lower H<sub>2</sub>O<sub>2</sub> % age indicated strong free radical scavenging activity.

Percent scavenging of the DPPH free radical was measured using the following equation (Avind *et al.*, 2010):

%DPPH radical scavenging (or % DPPH inhibition =  $[1/(As/Ac)] \times 100$ )

Here Ac = absorbance of control, As = absorbance of sample solution.

#### Preliminary phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures (Sofowra, 1993; Trease and Evans, 1989; Harborne, 1973).

#### Chromatographic method and isolation of lead compounds

The extractions of *Swertia chirayita* samples were carried out at room temperature by macerating the coarsely powdered plant materials (5g) with methanol and petroleum ether and occasionally shaking the contents. The extracts were concentrated under vacuum in a rotary evaporator (Eyela, NE-10, Japan). The extracts of petroleum ether were not taken up further for making fractions, as the weight of residue was negligible. The residues from methanolic extracts of three species were sequentially partitioned with hexane, chloroform and ethyl acetate to separate compounds of different polarities and to develop TLC fingerprint profile (Karan *et al.*, 2012).

#### STATISTICAL ANALYSIS

All the data was assessed by means of T test and Analysis of Variance (ANOVA) under completely Randomized Design (CRD) and the means of treatment were compared by Duncan's Multiple Range Test (DMRT) (Steel *et al.*, 1997).

#### RESULTS

##### Hepatoprotective effect of the plant

The effects of the various fractions of *S. Chirayita* on serum alkaline phosphatase, transaminase and bilirubin levels in CCl<sub>4</sub>-induced liver damage in mice are abridged in table 1. Administration of CCl<sub>4</sub> resulted to a significant (P<0.05) elevation of hepatospecific serum markers ALT, AST, ALP and bilirubin in CCl<sub>4</sub>-treated group (Group II), in comparison with the normal control group (Group I). On administration of the test fractions (Group IV to VIII) and silymarin (Group III) the level of some of these enzymes were found to decrease, especially NCM methanolic 300mg/kg and NCK methanolic 300mg/kg

were found the leading extracts for being excellent hepatoprotective.

The results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animals exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus, nucleolus and well brought out central vein (fig. 1-A), whereas that of CCl<sub>4</sub> intoxicated group animals showed total loss of hepatic architecture (fig. 1-B). There is also a micro view of liver tissue treated with Silymarin (fig. 1-C). Treatment with methanolic and aqueous extracts of *Swertia Chirayita* b.wt also showed moderate activity in protecting the liver cells from CCl<sub>4</sub> injury (Fig. D-I). The 300mg/kg dose of NCM and NCK (methanolic extracts) were found more effective in controlling the toxic effects of carbon tetrachloride and are depicted in fig. D-F. The treatment with *Swertia chirayita* extracts returned the injured liver towards normal side. Histopathological studies further revealed the hepatoprotective effects of whole plant of *Swertia chirayita*.

#### Antioxidant effect of the plant

The present study revealed reducing power of 80% methanolic extracts of NCM, NCK and ICM, represented as milligram of ascorbic acid equivalent per gram of dry weight. At concentration of 35µg/ml; NCM, NCK and ICM significantly showed absorbance  $0.198 \pm 0.003$ ,  $0.201 \pm 0.005$  and  $0.203 \pm 0.015$  respectively. Absorbance by ascorbic acid as standard was found to be  $0.766 \pm 0.047$ . The effects of *Swertia chirayita* (three dosages) comparable to those of Vitamin C as shown in table 2, except in MDA level in the liver and GSH level in the kidney ( $p < 0.05$ ). It suggests that the methanolic extracts of *Swertia chirayita* possess *in vitro* and *in vivo* antioxidant effects.

At concentration of 35µg/ml; NCM, NCK and ICM significantly scavenged H<sub>2</sub>O<sub>2</sub> ( $51.48 \pm 2.88$ ,  $51.22 \pm 2.44$ ,  $51.36 \pm 2.60$  respectively) and %age hydroxyl radical inhibition by ascorbic acid as standard was found to be  $89.95 \pm 0.92$ . The radical scavenging activity of *Swertia Chirayita* extract as compared to ascorbic acid is shown in table 3. It has been shown that the reducing power and scavenging effects on H<sub>2</sub>O<sub>2</sub>, increases sharply with the increasing concentration of the samples and standards to certain extend.

#### Phytochemical constituents of the plant

Preliminary phytochemical tests revealed that, Alkaloids, flavonoids, saponins, ascorbic acid, glycosides, steroids and triterpenoids were present in all three species of plant. Tannins and polyphenols were found completely absent in all, but anthraquinones were present in NCK, and absent in NCM and ICM.

#### Thin layer chromatography

TLC was performed with different solvent systems and found three different compounds on the basis of R<sub>f</sub> values, which are 0.34, 0.38 and 0.5 respectively. Of the assorted solvent systems, toluene: acetone (9.5:0.5) was finally declared as best solvent system for preparing TLC fingerprint profile and among three fractions, n-hexane fraction showed best resolution of spots. Some of the best TLC results were shown in fig. 2 (a, b, c). Further studies are needed to elucidate their structure and their possible role in various pharmacological activities of *Swertia chirayita*.

#### Toluene: acetone (9.5:0.5)

No spot is seen, each with chloroform and ethyl acetate fractions, 7 spots were revealed with n-hexane fraction (R<sub>f</sub> = 0.01, 0.06, 0.13, 0.19, 0.27, 0.38, 0.52).

#### DISCUSSION

Liver cells participate in metabolic activities and contain host of enzymes. In tissue, AST subsists in mitochondria and asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were found to be in greater concentrations in cytoplasm. In liver injury, transport function of the hepatocytes gets disturbed, ensuing in the outflow of plasma membrane and thereby creating an increased enzyme level in serum (Weber *et al.*, 2003). The raised activities of these enzymes are symbolic of cellular leakage and the functional integrity of the cell membranes in liver. ALP is excreted by liver through bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile by the liver and is reflected in their increased levels in serum. The present study was carried out to find out the effect of the formulation on the, CCl<sub>4</sub>-induced hepatotoxicity.

It is well recognized that hepatotoxicity by CCl<sub>4</sub> is due to enzymatic activation to free CCl<sub>3</sub> radical in free state, which in turn disturbs the function and structure of lipid and protein macromolecule in the membrane of the cell (Galelli and Castro, 1998). The steadiness of serum bilirubin, AST, ALT and ALP levels by herbal formulation is a clear sign of the perfection of the functional status of hepatic cells. The raised levels of these enzymes are the conventional indicator of liver injury. In the current study, a significant ( $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.05$ ) reduction in the level of serum bilirubin, ALP, ALT, AST was observed in the groups of animals treated with 200 and 300 mg/kg, b.w of herbal formulation. Silymarin significantly ( $P < 0.01$ ) reduced these levels to normal (table 1). The results reveal the hepatoprotective effect of herbal formulation compared with the CCl<sub>4</sub>-treated group. This finding can further supported with histopathological studies. The histopathological examination clearly reveals that the hepatocytes, central vein, are almost normal in herbal

formulations 200 and 300 mg/kg b.w, aqueous and Methanolic extracts, treated group in contrast to the toxicant group (fig. 1).

The comparative study between three species of *Swertia Chirayita* showed that NCM methanolic 300mg/kg and NCK methanolic 300 mg/kg were found the leading extracts for being excellent hepatoprotective, as indicated by the lowering down of serum enzymes and plasma bilirubin levels as compared to these parameters in  $\text{CCl}_4$  induced mice. The hepatoprotection exhibited by the extracts against  $\text{CCl}_4$  induced hepatic injury in animals also confirm the conventional use of *Swertia Chirayita* in hepato-biliary disorders.

The plant extracts were assessed for the occurrence of diverse groups of chemical constituents and they were found to hold alkaloids, flavonoids, saponins, ascorbic acid, glycosides, steroids and triterpenoids. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). The plant extracts were also revealed to contain saponins, which are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000; Okwu and Okwu, 2004). Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.*, 1994). Several workers have reported the analgesic antispasmodic and antibacterial properties of alkaloids (Stray, 1998). Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy, 1990).

We have found alkaloids, flavonoids, saponins, ascorbic acid, glycosides, steroids and triterpenoids in all three-plant samples of *Swertia chirayita*. Tannins and polyphenols were found completely absent in all, but anthraquinones were present in NCK, and absent in NCM and ICM. The results obtained in this study suggested that, identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Numerous antioxidants mainly come from plants in the form of phenolics compounds (flavonoids, phenolic acids

and alcohols, tocopherols, tocotrienols) ascorbic acid and caretonoids (Qureshi *et al.*, 2010). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen ion donor, and oxygen singlet oxygen acceptors. Reducing power assay involved  $\text{FeCl}_3/\text{K}_3\text{Fe}(\text{CN})_6$  complex as a source of ferric ions, which may reduce to ferrous ions in the presence of certain phytochemicals such as phenolics and flavonoids. These phytochemicals donate their hydrogen to  $\text{Fe}^{+3}$  ions and convert them to reduced form  $\text{Fe}^{+2}$  resulting in the production of intense green color and greater absorbance. Scavenging of extracts may be attributed to the presence of high phenolics contents, which donate their electrons to  $\text{H}_2\text{O}_2$ , thus neutralizing to water. Although  $\text{H}_2\text{O}_2$  itself is not very reactive but it may cause toxicity by giving rise the hydroxyl radicals level in the cell. Among reactive oxygen species (ROS), the hydroxyl radical is considered as the most reactive which by abstracting hydrogen from adjacent molecules induces severe damage to membrane lipid, causes lipid peroxidation and cellular toxicity (Yen and Duh, 1993).

Our study revealed reducing power of 80% methanolic extracts of NCM, NCK and ICM, represented as milligram of ascorbic acid equivalent per gram of dry weight. At concentration of  $35\mu\text{g/ml}$ ; NCM significantly showed better absorbance than NCK and ICM. Absorbance by ascorbic acid as standard was found to be  $0.766\pm0.047$ . It suggests that the methanolic extracts of *Swertia chirayita* possess *in vitro* and *in vivo* antioxidant effects. At concentration of  $35\mu\text{g/ml}$ ; NCM, again significantly scavenged  $\text{H}_2\text{O}_2$  and %age hydroxyl radical inhibition by ascorbic acid as standard was found to be  $89.95\pm0.92$ . It has been shown in the results that the reducing power and scavenging effects on  $\text{H}_2\text{O}_2$ , increases sharply with the increasing concentration of the samples and standards to certain extend.

Thin layer Chromatography (TLC) is a simple, low-cost, versatile and specific method for the identification of herbal medicines. The unique feature of picture-like image of TLC supplies an intuitive visible profiling (Shaw and Butt, 1995). TLC is an important tool, not only for the quality control of medicinal plants, but also for the analysis of herbal drugs (Durón *et al.*, 2009). It is a popular method to analyze plant drugs and has been used for a wide variety of applications. In several pharmacopoeias, a great variety of plants are identified by the use of TLC methods (Renato *et al.*, 2012). TLC was chosen, because this separation system enables simultaneous evaluation of standards and samples, thus matching the working conditions and environment for both, and reducing systematic errors (Kaiser, 2005). The appearance of compounds in common and different bands can be useful for identification and authentication of plant drugs.

**Table 1:** Effects of *Swertia Chirayita* on serum ALT, ALP, AST and Bilirubin activities

| Sample            | Biochemical Parameters  |                                |                          |                           |
|-------------------|-------------------------|--------------------------------|--------------------------|---------------------------|
|                   | Bilirubin (U/L)         | ALT (U/L)                      | ALP (U/L)                | AST (U/L)                 |
| Normal            | 0.60033±0.010028        | 47.2633±7.0964                 | 135.54±2.435             | 41.6266±3.084             |
| CCL <sub>4</sub>  | 1.3673±0.03754          | 216.52±2.671                   | 638.0167±5.678           | 171.711±3.8799            |
| Silymarin         | 0.211±0.05808           | 62.456± 34.869*                | 283.983±22.398           | 50.6433±4.582             |
| NCM meth 200mg/kg | 1.0121±0.0371           | 163.35±5.6811                  | 528.77± 5.2630           | 131.475±2.386 $\alpha$    |
| NCM meth 300mg/kg | 0.875±0.0094*           | 106.521±5.4977*                | 430.198±6.3882           | 97.018±4.658* $\beta$     |
| NCM aq 200mg/kg   | 1.2068±0.04566 $\alpha$ | 165.186±3.9961 $\alpha\lambda$ | 571.465±3.6582 $\lambda$ | 190.1083±3.5451           |
| NCM aq 300mg/kg   | 1.05683±0.0598          | 133.35±3.7427                  | 545.27±6.0308            | 170.18±3.638 $\lambda$    |
| ICM meth 200mg/kg | 1.11083± 0.03921        | 169.255±3.0158 $\lambda\alpha$ | 529.50±4.9344            | 130.808±2.095             |
| ICM meth 300mg/kg | 0.8666± 0.0448* $\beta$ | 145.688± 4.9562                | 496.86± 5.459            | 100.878±3.083*            |
| ICM aq 200mg/kg   | 1.180±0.027 $\lambda$   | 159.25±4.8599                  | 569.798±5.677 $\alpha$   | 180.9733±4.707 $\lambda$  |
| ICM aq 300mg/kg   | 0.98266±0.02825*        | 135.1183±3.82719               | 540.846±7.797            | 155.108±8.986             |
| NCK meth 200mg/kg | 1.04533±0.0538          | 159.813±3.8643                 | 532.175±4.522            | 129.4533±3.4209           |
| NCK meth 300mg/kg | 0.874±0.0108* $\beta$   | 105.02± 4.527*                 | 426.69±4.8885            | 97.685±2.9076* $\beta$    |
| NCK aq 200mg/kg   | 1.0171±0.0394           | 132.355±4.3422                 | 542.276±6.4237           | 166.636±4.47918 $\lambda$ |
| NCK aq 300mg/kg   | 1.186±0.0275 $\lambda$  | 165.73±5.727                   | 568.1483±3.039 $\alpha$  | 188.281±4.6753            |

Data are expressed as mean  $\pm$  S.D (n=6)

Values significantly different compared to normal control: \*P<0.05

Values significantly different compared to CCL<sub>4</sub>-administered control:  $\alpha$ P<0.05,  $\lambda$ P<0.01

Values significant varied comparable to standard drug:  $\beta$ P< 0.001

**Table 2:** Reducing power assay of methanolic extracts of *Swertia Chirayita*

| Test   | Reducing power assay (absorbance at 700nm) |             |             |             |                   |
|--------|--------------------------------------------|-------------|-------------|-------------|-------------------|
| Sr. no | Plant extracts Conc. $\mu$ g/ml            | NCM         | NCK         | ICM         | Ascorbic acid     |
| 1      | 5 $\mu$ g/ml                               | 0.071±0.005 | 0.076±0.003 | 0.078±0.008 | 0.248±0.012       |
| 2      | 10 $\mu$ g/ml                              | 0.081±0.006 | 0.086±0.005 | 0.089±0.003 | 0.498±0.020       |
| 3      | 15 $\mu$ g/ml                              | 0.104±0.002 | 0.108±0.006 | 0.114±0.005 | 0.524±0.029       |
| 4      | 20 $\mu$ g/ml                              | 0.141±0.015 | 0.164±0.009 | 0.149±0.017 | 0.546±0.012       |
| 5      | 25 $\mu$ g/ml                              | 0.151±0.003 | 0.154±0.015 | 0.159±0.007 | 0.590 $\pm$ 0.056 |
| 6      | 30 $\mu$ g/ml                              | 0.166±0.015 | 0.164±0.008 | 0.168±0.007 | 0.658±0.018       |
| 7      | 35 $\mu$ g/ml                              | 0.198±0.003 | 0.201±0.005 | 0.203±0.015 | 0.766±0.047       |

Values are in terms of Mean  $\pm$  SD after triplicate analysis

**Table 3:** Scavenging potential of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by NCM, NCK, ICM

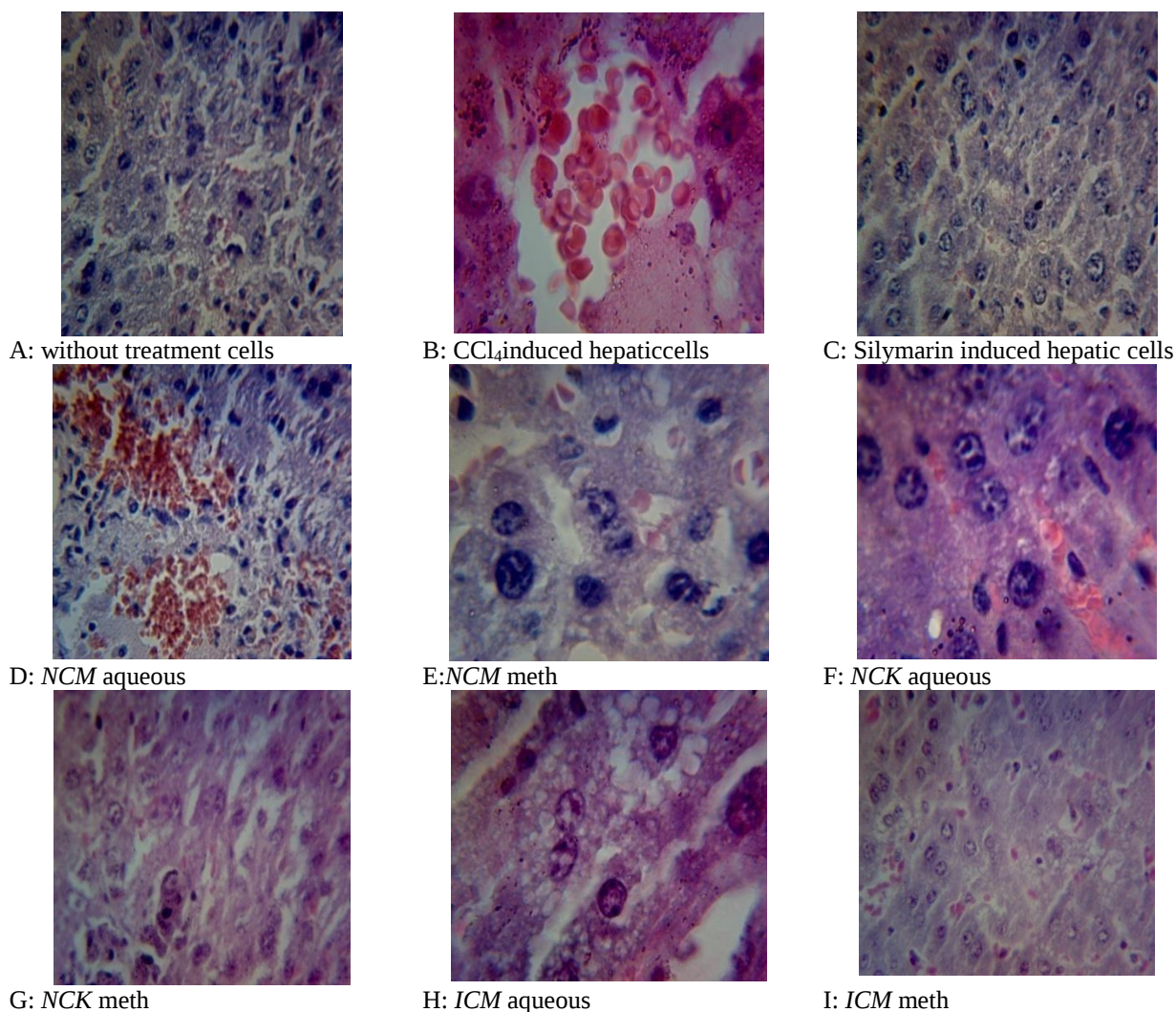
| S. No. | Extract conc. ( $\mu$ g/ml) | % age inhibition of H <sub>2</sub> O <sub>2</sub> |             |             | Ascorbic Acid |
|--------|-----------------------------|---------------------------------------------------|-------------|-------------|---------------|
|        |                             | NCM                                               | NCK         | ICM         |               |
| 1      | 5 $\mu$ g/ml                | 7.51±2.94                                         | 7.21±2.64   | 7.38±2.70   | 13.69±0.2     |
| 2      | 10 $\mu$ g/ml               | 12.23±0.56                                        | 11.98±0.38  | 12.06±0.56  | 20.20±0.3     |
| 3      | 15 $\mu$ g/ml               | 17.95± 0.42                                       | 17.52±0.54  | 17.76± 0.48 | 46.80±0.8     |
| 4      | 20 $\mu$ g/ml               | 20.08± 1.60                                       | 19.28± 1.68 | 20.48± 1.66 | 61.72±11      |
| 5      | 25 $\mu$ g/ml               | 33.89± 0.56                                       | 32.94± 0.76 | 33.66± 0.58 | 70.25±0.7     |
| 6      | 30 $\mu$ g/ml               | 45.90±0.84                                        | 44.98±0.74  | 45.80±0.74  | 80.58±0.2     |
| 7      | 35 $\mu$ g/ml               | 51.48±2.88                                        | 51.22±2.44  | 51.36±2.60  | 89.95±0.2     |

Values expressed as mean  $\pm$  SD, (n=3)

## CONCLUSION

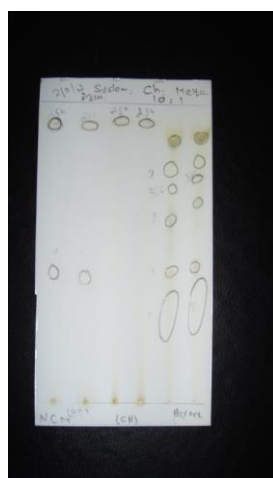
The comparative study between three species of *Swertia chirayita* extracts collected from various places at different doses revealed that NCK was found best being having better fraction of phytochemical constituents as compared to the other two species. NCM is noted to best for being a good antioxidant, and also having a finest

Chromatographic profile (TLC) as it shows the R<sub>f</sub> values of all three known isolated compounds with different solvent systems., where as NCM methanolic 300mg/kg and NCK methanolic 300mg/kg were found the leading extracts for being excellent hepatoprotective, as indicated by the lowering down of serum enzymes and plasma bilirubin levels as compared to these parameters in CCL<sub>4</sub> induced mice.



**Fig. 1:** Histopathological Examination of hepatic cells under various treatments

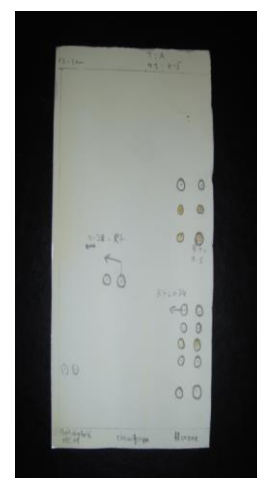
A: hepatic cells without any treatment, B:  $\text{CCl}_4$  induced hepatic cells, C: Silymarin induce hepatic cells, D and E: cells treated by aqueous and methanolic extracts of *NCM*, F and G: cells treated by aqueous and methanolic extracts of *NCK*, H and I: cells treated by aqueous and methanolic extracts of *ICM*.



2a: Chloroform: Methanol system  
**Fig. 2:** TLC pattern of *NCM* extract



2b: Toluene: Ethyl acetate: Formic acid



2c: Toluene: Acetone system

## ACKNOWLEDGEMENTS

We are very grateful to staff of Animal house and Chemical Research laboratory, Drugs control and traditional medicines division, National Institute of Health, Islamabad, Pakistan for their help and support

## REFERENCES

- Ahsan R, Islam M, Bulbul JI, Musaddik A and Haque (2009). Hepatoprotective activity of ethanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Eur. J. of Sci. Res.*, 7(2): 302-310.
- Alam DK, Ali SK, Mahjabeen S, Hassan R, Rahman F and Chowdhury RMAA (2001). Potential hypoglycemic effects of swertia chirata – An indian subcontinental herb with important medicinal value. *Pharmacology online*, 2: 642-647.
- Alam MS, Kaur G, Jabbar Z, Javed K and Athar M (2007). *Eruca sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity, *Food Chem. Toxicol.*, 45: 910-920.
- Alvari A, Ohadi Rafsanjani MS, Ahmad FJ and Abdin MZ (2012). Current status and future prospects of hepatoprotective herbal medicines: Contemporary Overview into the Clinical Trials, *Rev. Recent Clin. Trials.*, 7(3): 214-235.
- Avind KG, Sushil KM and Arnab S (2010). Evaluation of the DPPH radical scavenging activity, total phenols and anti-oxidant activities in 5 Indian wild bambusa vulgaris “Vittata” methanolic leaf extract. *J. Nat. Pharm.*, 1(1): 40-45.
- Durón RR, Almaguer LC, Garza-Juárez AJ, Cavazos MLS and De-Torres NW (2009). Development and validation of thin-layer chromatographic methods for quality control of herbal products, *Acta Chromatog*, 21: 203-215.
- Galelli ME and Castro JA (1998). Effect of trichloromethyl and trichloromethyl peroxy free radicals on protein sulfhydryl content studies in model and in enzymatic carbon tetrachloride activation systems. *Res. Commun. Mol. Pathol. Pharmacol.*, 100(2): 227-238.
- Harborne JB (1973). Methods of plant analysis, In: *Phytochemical Methods*, Chapman and Hall, London. pp. 49-188.
- Hussain S, Malik F, Khalid N, Qayyum MA and Riaz H (2012). Alternative and traditional medicines systems in Pakistan: History, Regulation, Trends, Usefulness, Challenges, Prospects and Limitations, A Compendium of Essays on Alternative Therapy, Arup Bhattacharya (Ed.), ISBN: 978-953-307-863-2. 1st ed. In Tech., Pp. 89-108.
- Just MJ, Recio MC, Giner RM, Cueller MU, Manez S, Billia AR and Rios JL (1998). Antiinflammatory activity of unusual lupine saponins from *Bupleurum Fruticescens*. *Planta Medica.*, 64(5): 404-407.
- Kaiser ER (2005). Methods of detecting and/or reducing systematic errors in quantitative planar chromatography. Part 3. evaluation and calibration errors. *J. Planar Chromatogr.*, 18: 256-263.
- Kaplowitz N (2001). Drug-induced liver disorders: implications for drug development and Regulation, *Drug Saf.*, 24(7): 483-490.
- Karan M., Jamwal A., and Vasisht K (2012). Phytochemical studies on swertia chordata (G. Done) clark and comparative chromatographic evaluation of Five Different Species of Swertia. *IJAHM*, 2(2): 294-299.
- Khan RA, Khan MR and Sahreen S (2010). Evaluation of *Launaea procumbens* in renal disorders: A rat model. *J. Ethnopharmacol.*, 128: 452-461.
- Kristina MM, Madeleine T, Katrin F, Jan-Wilhelm K, Harald E, Jan PT and Richard M (2012). Hepatic growth hormone and glucocorticoid receptor signaling in body growth, steatosis and metabolic liver cancer development. *Mol. Cell. Endocrinol.*, 25: 361(1-2): 1-11.
- Lim YY and Murtijaya J (2007). Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT*. 40: 1664-1669.
- Marjorie C (1996). Plant products as anti-microbial agents. *Clinical Microbiol. Rev.*, 12: 564-582.
- Michael PH and Cynthia J (2006). Mechanisms of drug-induced liver injury. *AAPS Journal*, 8(1): E48-E54.
- Murugesan GS, Sathishkumar M, Jayabalan R, Binupriya AR, Swaminathan K and Yun SEZ (2009). Hepatoprotective and curative properties of Kombucha tea against carbon tetrachloride-induced toxicity, *J. Microbiol. Biotechnol.*, 19: 397-402.
- Nobori T, Miurak K, Wu DJ, Takabayashik LA and Carson DA (1994). Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature.*, 46: 753-756.
- Nyarko AA and Addy ME (1990). Effects of aqueous extract of *Adenia cissampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Res.* 4(1): 25-28.
- Okwu DE (2001). Evaluation of chemical composition of medicinal plants belonging to Urophorbiaceae. *Pak. Vet. J.*, 14: 160-162.
- Okwu DE and Okwu ME (2004). Chemical composition of *Spondias mombin* linn. plant parts. *J. Sustain. Agric. Environ.*, 6(2): 140-147.
- Qureshi R, Bhatti GR and Memon RA (2010). Ethnomedicinal uses of herbs from northern part of Nara desert, Pakistan. *Pak. J. Bot.*, 42: 839-851.
- Raquel FE (2007). Bacterial lipid composition and antimicrobial efficacy of cationic steroid compounds. *Biochemica et Biophysica Acta.*, 2500-2509.
- Renato B, Luciana G.W, Gisely C. L, João CP de Mello (2012). Quality control and TLC profile data on

- selected plant species commonly found in the Brazilian market. *Braz. J. Pharmacog.* **22**(5): 1111-1118.
- Saha P and Das S (2001). Regulation of hazardous exposure by protective exposure- Modulation of phase II detoxification and lipid peroxidation by *Camellia sinensis* and *Swertia chirata*. *Teratog Carcinog Mutagen. Suppl 1*, pp.313-322.
- Saller R, Meier R and Brignoli R (2001). The use of silymarin in the treatment of liver diseases, *Drugs*, **61**: 2035-2063.
- Shanmugasundaram P and Venkataraman S (2006). Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract, *J. Ethnopharmacol.*, **104**: 124-128.
- Shaw PC and Butt PP (1995). Authentication of panax species and their adulterants by random primed polymerase chain reaction, *Planta Med.*, **61**: 466-469.
- Sodipo OA, Akiniyi JA and Ogunbamosu JU (2000). Studies on certain on certain characteristics of extracts of bark of *Pansinystalia macruceras* (K schemp) picrre Exbeille. *Global J. Pure Appl. Sci.*, **6**: 83-87.
- Sofowra A (1993). Medicinal plants and traditional medicine *In: Africa*. spectrum books Ltd., Ibadan, Nigeria. Pp.191-289.
- Steel RGD, Torrie JH and Dickey DA (1997). Principles and procedures of statistics. Biometrical approach. 3<sup>rd</sup> Ed., McGraw Hill Book Co., New York, USA, pp.400-428.
- Stray F (1998). The natural guide to medicinal herbs and plants. tiger books international, London. Pp.12-16.
- Tasduq SA, Peerzada K, Koul S, Bhat R and Johri RK (2005). Biochemical CCl<sub>4</sub> (tetrachloride) induced hepatotoxicity in rats. *African J. Pharma. Pharmacol.*, **4**: 731-734.
- Trease GE and Evans WC (1989). Pharmacognosy 2<sup>nd</sup> Edn. Braille Tiridel and Macmillan Publishers, London.
- Weber LW, Boll M and Stampfl A (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.*, **33**(2): 105-136.
- World Health organization (2000). Hepatitis B (Fact sheet No. 204). Geneva, Switzerland.
- Wojdyło A, Oszmianski J and Czemerys R (2005). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* **105**: 940-949.
- Yen P and Duh D (1993). Anti-oxidant properties of methanolic extracts from peanut hulls. *J. Ame. Oil. Chemist's Soc.*, **70**: 383-386.
- Zimmerman HJ (1978). Drug-induced liver disease. *Drugs*, **16**(1): 25-45.