IJPSR (2013), Vol. 4, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 December, 2012; received in revised form, 08 February, 2013; accepted, 29 March, 2013

HEPATOPROTECTIVE ACTIVITY OF *SCINDAPSUS OFFICINALIS* FRUIT IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

Nikhil Shrivastava*[1], Preeti Agrawal [2], Akanksha Khare [3], Sarlesh Rajput [2], Rajendra Singh Baghel [1], Satyendra Singh Baghel [1]

Department of Pharmacology (Toxicology Research Division) [1], Department of Pharmaceutics [2], Shriram College of Pharmacy, S.R.G.O.C. Campus, AB Expressway Banmore, Madhya Pradesh, India
Community Health Center [3], Gaurihar Madhya Pradesh, India

Keywords:
Scindapsus officinalis (Roxb.), Cytoprotection, Hydroalcoholic extract, SGPT, SGOT, ALP

Correspondence to Author:

Nikhil Shrivastava

Department of Pharmacology (Toxicology Research Division), Shriram College of Pharmacy, S.R.G.O.C. Campus, AB Expressway Banmore, Madhya Pradesh, India

E-mail: nikhil.pharma@live.com

ABSTRACT: *Scindapsus officinalis* (Roxb.) Schott fruits are widely used in many parts of India for the treatment of various diseases. It is one of the plants used in Indian system of medicine which belongs to family Aracea. It has the significant antioxidant property due to presence of flavonoids and phenolic compound and has ability of cytoprotection due to antioxidant property. Hydroalcoholic extract (50%Ethanol) of *Scindapsus officinalis* fruit was prepared and evaluated for its hepatoprotective Potential against paracetamol-induced hepatotoxicity in rats. Alteration in the levels of SGPT, SGOT, ALP, bilirubin, total protein and tissue GSH, GSSG and MDA were tested in both treated and untreated groups. SGPT, SGOT, ALP and bilirubin was enhanced significantly ($p<0.05$) in Paracetamol (2mg/kg B.wt.) treated group and total serum protein, tissue MDA rise and tissue level of GSH was significantly ($p<0.05$) reduced in Paracetamol (2mg/kg B. wt.) treated group. Pretreatment with Hydroalcoholic extract (50% Ethanol) of *Scindapsus officinalis* fruit (200mg/kg B. wt. and 400 mg/kg B. wt.) has brought back the altered levels of biochemical markers to the near normal levels. The histopathology of the liver tissue shows liver necrosis and the recovery is significant in HESO treated groups. Silymarin is used as standard drug. All statistics was done on SPSS windows Ver.16.0.

INTRODUCTION: *Scindapsus officinalis (Roxb.)* Schott. is one of the plant used in Indian system of medicine which belongs to family Araceae. The plant of *Scindapsus officinalis* is a large, stout, epiphytic and perennial climber with adventitious aerial roots growing on trees and rocks [1-3].

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Article can be accessed online on:
www.ijpsr.com

The plant is growing in tropical part of India. It is common in the Midnapore district of west Bengal and cultivated vegetatively for its fruit, which is cut into transverse pieces, dried and used medicinally [4-6].

Fruit is very important part of the plant and accepted as raw drug of known properties in both Ayurvedic and Unani system of medicine. The fruit is reported to be useful as a diaphoretic, carminative stimulant, anthelmintic aphrodisiac, galactagogue, appetizer and also useful in the form of decoction in diarrhea, asthma and other affections supposed to be caused by Kafa [7-10].

Anatomical/histological practice playing a unique role in the more detailed examination of crude drugs and can be used to confirm the structural features of the crude drugs. Quantitative microscopy and linear measurements are the other important aspects of the histological method [11]. The histological approach to study plants and plants parts is helpful in the searching of specific microscopical characters and even some times it is helpful in the differentiation between two species of same genus. Based on this fact and since no complete anatomical data related to fruit is available so far.

The plant of *Scindapsus officinalis* is large climber with areal roots growing on trees and rocks. The fruit of *Scindapsus officinalis* is known as Gajpeepal in ayurveda. Gajpeepal consists of dried, transversely cut pieces of mature female spadix of *Scindapsus officinalis* (Fam. Araceae). It is found all along the sub-Himalayan tract between an altitude of 330-1000 m in West Bengal, Orissa, Andhra Pradesh and the Andaman Islands. *Scindapsus officinalis* have some therapeutic activities which are pharmacologically aprooved i.e. antioxidant activity, anti- inflammatory and analgesic, antihistaminic activity, antibacterial activity, antidiabetic activity [12].

It has the significant antioxidant property due to presence of flavonoids and phenolic compound and has ability of cytoprotection due to antioxidant property [13].

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins [14].

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures [14].

Scindapsus officinalis consists of secondary metabolites like flavonoids, tannins, glycosides, alkaloids, terpenes, etc. The folk lore claim of *Scindapsus officinalis* fruits are antidiabetic, anthelmintic, aphrodisiac, galactagogue, stimulant, diaphoretic, antidiarrhoeal, carminative, expectorant, tonic, antiprotozoal, anticancer, sharpening hearing, aphrodisiac, cardiotonic and regulating the bowel and appetite. It is also used in dysentery,asthma, troubles of the throat, rheumatism, asthma, worm infestations, pharyngopathy, helminthiasis and bronchitis. There is scientific report for the evaluation of Hepatoprotective activity in *Scindapsus officinalis* fruit.

Hence, the objective of the present study to investigate the Hepatoprotective Activity of *Scindapsus Officinalis* fruit in Paracetamol induced Hepatotoxicity in Rats.

MATERIAL & METHODS:

Collection of plant material: The *Scindapsus officinalis* fruit will be collected from the local market of Haridwar, Uttarakhand state, India fruits are Identified and authenticated by Dr. Anil Mangal (Research officer Ayurveda) National Research institute for Ayurveda HRD, Gwalior (M.P). The voucher specimen no:srcp/2012/002 has been deposited at the herbarium unit of the department of pharmacognosy, Shriram college of pharmacy Banmore , morena.

Preparation of extract: Fruits were shade dried and convert into course powder by using hand grinder and then sieved the powder by a sieve mesh size 12 for uniform particle size powder. Extract was prepared by cold maceration method. About 600 gm. of Powdered material extract with hydro alcoholic solvent (50% ethanol) with quantity of 4.00 liter for 72 hrs for extraction.

After complete extraction the extract was concentrated by distilling of the solvents and then evaporated to dryness on water bath color of extract was observed and percentage yield was calculated on the air dried basis.

Type of extract	Color	Percentage yield
50% ethanolic extract	Brown	4.83%

Drugs & chemicals: Paracetamol (to cause hepatic injury), Silymarin (standard reference). Ether, Ethanol, Formaline, Liquid paraffin, Hematoxyline, Eosin. KCl, TriChloroacetic acid, EDTA, Tris HCl buffer, DTNB, NEM, Thiobutyric acid etc.

Experimental Animals & Procedures:

Animals: Male wistar rats weighing between 200-220 gm were used for this study. The animals were obtained from central animal facility of ShriRam College of Pharmacy, Banmore, M.P., India [891/AC/05/CPCSEA] and was maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2^{o}C) and acclimatized to 12/12 h light/dark cycle. Free access to food and water was allowed until 2h before the experiment. The care and maintenance of the animals was as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation

Hepatoprotective activity: A total of 30 animals were equally divided into five groups (n=6) by randomization.

Group –I: Naive control (0.5% CMC 1 ml/kg b.wt., oral)

Group –II: PCM (2mg/kg b.wt., oral)

Group -III: Silymarin (100mg/kg b.wt., oral) + PCM (2mg/kg b.wt., oral)

Group –IV: HESO (200mg/kg b.wt., oral) + PCM (2mg/kg b.wt., oral)

Group –V: HESO (400mg/kg b.wt., oral) + PCM (2mg/kg b.wt., oral)

Animals were divided into five groups of 6 animals each. The first group received saline 1 ml/kg for one week (control). The group II received 0.5% CMC 1 ml/kg for one week (positive control). The groups III, IV and V received 200 mg/kg and 400 mg/kg of Hydro alcoholic extract of *Scindapsus officinalis* fruit and silymarin (100 mg/kg p.o.) respectively once a day for seven days.

On the fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV and V were administered with paracetamol 2 g/kg orally. The body weights of the animals were also recorded daily up to 7 days. After 7 days animals was anaesthetized with ether for collection of blood from retro orbital plexus, and then sacrificed by cervical dislocation for the removal of liver. Various hematological and biochemical analysis were carried out.

Biochemical evaluation in Serum:

1. **Serum Marker Enzymes Estimation**: Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP), Total protein concentration and total bilirubin was estimated by using commercial kits (E- coline, Merck, India) as per the manufacturer instructions.

2. **MDA assay:** The reaction mixture containing 1ml 0.67% thiobarbituric acid (TBA), 1 ml 20% trichloro acetic acid (TCA), and 100 µl serum was incubated at 100^{o}C for 20 min and centrifuged at 12,000 rpm for 5 min. The absorbance of the supernatant was read at 532 nm and MDA concentration was determined by using a molar extinction coefficient of 1.56×10 ^{5}/M/cm and the values was expressed as mM [15].

Biochemical evaluation in liver tissue:

1. **Lipid Peroxidation Assay:** A portion of the liver was used for biochemical estimation. Liver lipid peroxidation was determined by measuring the level of MDA according to the method of Ohkawa *et al*., 1979. 2ml of suspension medium was taken from the supernatants of the 10% tissue homogenate in 1.15% KCl and centrifuged at 10,000 rpm. 1ml of 30% TCA followed by 1ml of 0.8% TBA was added to it.

   The tubes was covered with aluminium foil and kept in shaking water bath for 30 minutes at 80^{0} C, after 30 min; tubes was taken out and kept in ice cold water for 10 min. They were then centrifuged at 3000 rpm for 15 min. The absorbance of supernatant was read at 540 nm at room temperature against blank. Blank consisted of 2ml distilled water, 1ml TBA, and 1ml TCA [16].

2. **GSH Assay:** Tissue GSH was determined by the method of Sedlak and Lindsay (1968). A portion of the reperfused liver tissue (300-600 mg) homogenized in 5-8 ml of 0.02M EDTA and then 4ml of cold distill water was added to it. After mixing 1ml of 50% TCA was added to it and shaken for 10min and centrifuged at 6000 rpm for 15 min. 4 ml of 0.4 M tris buffer was mixed with 2 ml of supernatant and 0.1ml of 0.01M DTNB. The absorbance of this resulting mixture was read at 410 nm at room temperature against reagent blank [17].

3. **Oxidized Glutathione (GSSG) Assay:** The samples were homogenized (1:20 w/v) in ice-cold 6% perchloric acid containing 20 mmol/l Nethymaleimide (NEM) and 2 mmol/l bathophenanthrolinedisul-fonic acid (BPDS). The homogenate was centrifuged at 15,000 x g for 5 min at 4°C and the supernatant neutralized before assay. Oxidized gluthione was measured according to the method described by Aseni *et al*. (1999) based on the principle of glutathione reductase enzyme reducing GSSG to GSH with the concomitant oxidation of NADPH to NADP+. To 0.9 ml of 1.75 mol/l K_3 PO_4 buffer (pH 7.0) containing 20 m mol/l NEM were added 0.05 ml of sample extract and 0.025 ml of 10 mg/ml of NADPH-Na solution. Absorbance at 340 nm was measured for 30 s immediately after addition of 0.025 ml of (10 mg/ml) Glutathione Reductase (GR) to the assay mixture [17].

Histopathology of liver:

A. **Tissue Fixation:** Liver tissues of rat was removed and washed with normal saline. Formaldehyde, as 4% buffered formaldehyde (10% buffered formalin), is the most widely employed universal fixative particularly for routine paraffin embedded sections. The cleared tissue was fixed in 10% natural buffered formalin solution (pH 7.0-7.2).

B. **Tissue Processing:** the aim of tissue processing is to embed the tissue in a solid medium firm enough to support the tissue and give it sufficient rigidity to enable thin sections to be cut , and yet soft enough not to damage the knife or tissue.

Stages of processing:

1. Dehydration.
2. Clearing.
3. Embedding.

1. **Dehydration:** Dehydration was done to remove fixative and water from the tissue and replace them with dehydrating fluid. There are a variety of compounds many of which are alcohols. Several are hydrophilic so attract water from tissue. To minimize tissue distortion from diffusion currents, tissues were dehydrated by using increasing strength of alcohol; e.g. 50%, 70%, 90% and 100%.

2. **Clearing:** In clearing, dehydrating fluid was replaced with a fluid that is totally miscible with both the dehydrating fluid and the embedding medium. Toluene was used for clearing the tissue.

3. **Embedding:** Embedding is the process, by which tissues are surrounded by a medium such as agar; gelatin or wax which when solidified will provide sufficient external support during sectioning. The tissue cassette Opened, mould was selected, and there should be sufficient room for the tissue with allowance for at least a 2 mm surrounding margin of wax. Then mould was filled with paraffin wax. Using warm forceps tissue was selected, taking care that it does not cool in the air; at the same time. Mould was chilled on the cold plate, orienting the tissue and firming it into the wax with warmed forceps. This ensures that the correct orientation is maintained and the tissue surface to be sectioned is kept flat. Block was removed from the mould.

C. **Cutting:** After hardening the tissues by replacing water with paraffin, the sections of 5 µ in thickness was cut with help of semi-automatic rotary microtome. From there the tissue can be mounted on a microscope slide, stained and examined using a light microscope.

D. **Staining:** H & E stains are universally used for routine histological examination of tissue sections. All the sections of the tissues were examined under microscope for the analyzing the altered architecture due to the liver tissue due to paracetamol challenge and improved liver

architecture due to pretreatment with test extracts and standard drug. These were examined under the microscope for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, Infiltration, kuffer cell and sinusoids and photographs was taken.

Organ to body weight indices (OBWI): After sacrificing the animals, liver will be removed and the washed free of extraneous materials and weighed. The organ to body weight indices (OBWI) will be calculated as per the formula given below:

$$\text{OBWI} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

Statistical analysis: Statistical evaluations were made using one-way ANOVA followed by Dunnet's test. A probability of $P \geq 0.05$ and less was taken as statistically significant. The Analysis was carried out using SPSS 16.0.

RESULT AND DISCUSSION:

1. **Percentage yield: Table 1** represents the yield of drug after extraction of powdered shade dried fruits of *scindapsus officinalis* with hydro alcoholic solvent (50% Ethanol) By Cold Maceration Method. Dried Extract is the dark brown color Powder.

TABLE 1: % YIELD AFTER EXTRACTION OF SHADE DRIED POWDERED FRUIT OF *SCINDAPSUS OFFICINALIS*

PARTICULARS	DESCRIPTION
Plant name	*Scindapsus officinalis*
Part used	Fruit
Solvent used	50% Ethanol
Weight of dried	600gms
Practical Yield	29gms
Percentage Yield	4.83%

The paracetamol-induced liver damage was treated with Hydroalcoholic extract of *Scindapsus officinalis* fruit for seven days continuously. The following observations were obtained.

2. **OBWI: Table 2 & fig. 2** represents the organ bodyweight indices of the different groups of rats. The OBWI of Paracetamol induced group II rats has been increased comparing with group I. After 7-days administration of HESO and silymarin on group III, group IV and group V rats has reached the OBWI almost to near normal level. The gain in body weight of group IV is compared with group II and it may be due to the regeneration of liver cells activity to near normal after the herbal treatment.

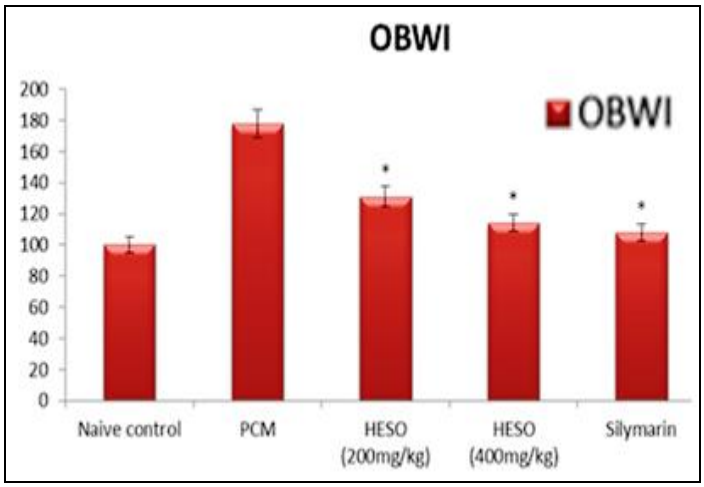


FIG. 2: EFFECT OF PCM, SILYMARIN AND HESO ON OBWI IN DIFFERENT ANIMAL GROUPS. Statistically Significance test was done by One Way ANOVA followed by Dunnett's 't' test. *P<0.05 compared to Disease control group. Control value for OBWI- 2.63 ± 0.012. All values are MEAN ± SEM of 6 animals per group.

TABLE 2: EFFECTS OF PCM, SILYMARIN AND HESO ON BODY WEIGHT, WET LIVER WEIGHT AND OBWI OF DIFFERENT ANIMAL GROUPS

S. no.	Groups	Treatment given	Body wt (gms) Mean±SEM	Wet liver wt (gms) Mean±SEM	OBWI Mean±SEM
1	Naive control	0.5% CMC (1 ml/kg)	200±2.03	5.26±0.04	2.63±0.012
2	Disease control	PCM (2mg/kg)	210±3.44	9.74±0.7	4.70±0.030
3	Test drug dose I	HESO (200mg/kg) + PCM (2mg/kg)	200±2.03*	6.90±0.57*	3.45±0.017*
4	Test drug dose II	HESO (400mg/kg) + PCM (2mg/kg)	210±3.44*	6.30±053*	3.00±0.016*
5	Standard Drug	Silymarin (100mg/kg) + PCM (2mg/kg)	205±1.23*	5.86±0.41*	2.85±0.013*

Statistically Significance test was done by One Way ANOVA followed by Dunnett's't' test using SPSS 16.0 Windows version. *P<0.05 compared to Disease Control group.

3. **Biochemical evaluation in serum**: **Table 3 & fig. 3** represents that the Paracetamol has enhanced the levels of SGPT, SGOT, serum alkaline phosphatase level (SALP), and table 4 & fig. 4 represents the bilirubin (both total and direct bilirubin levels) which also increased by PCM and significantly controlled by silymarin and HESO whereas plasma proteins are decreased significantly. This clearly indicates that there is a significant hepatic damage due to paracetamol. Treatment with silymarin and 200mg/kg and 400mg/kg of HESO has significantly brought down the elevated levels of SGPT, SGOT, SALP and bilirubin also significantly enhanced the decreased levels of plasma protein. Results are reported in tables.

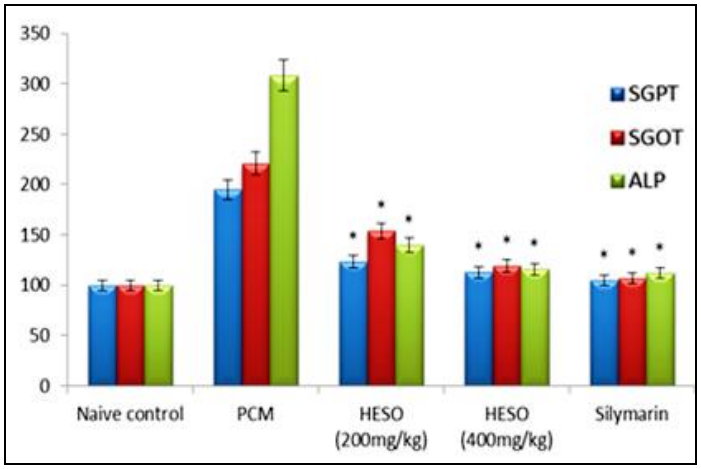


FIG. 3: EFFECT OF PCM, SILYMARIN AND HESO ON SERUM MARKERS (SGPT, SGOT AND ALP) IN DIFFERENT ANIMAL GROUPS. Statistically Significance test was done by One Way ANOVA followed by Dunnett's 't' test. *P<0.05 compared to Disease control group. Control value for SGPT- 69.3 ± 0.53, SGOT- 113.4 ± 5.56, ALP-159.3 ± 7.3. All values are MEAN ± SEM of 6 animals per group.

TABLE 3: EFFECTS OF PCM, SILYMARIN AND HESO ON SGPT, SGOT AND ALP OF DIFFERENT GROUPS OF ANIMALS

S. no.	Groups	Treatment given	SGPT Levels (U/L) Mean ± SEM	SGOT Levels (U/L) Mean ± SEM	ALP Levels (U/L) Mean ± SEM
1	Naive control	0.5% CMC (1 ml/kg)	69.3±3.53	113.04±5.56	150.3±7.3
2	Disease control	PCM (2mg/kg)	135.49±6.32	250.30±7.50	463±13.1
3	Test Drug dose I	HESO (200mg/kg) + PCM (2mg/kg)	85.57±3.30*	175.20±5.30*	210.6±2.21*
4	Test drug dose II	HESO (400mg/kg) + PCM (2mg/kg)	78.53±2.32*	135.93±3.48*	174.4 ± 5.1*
5	Standard Drug	Silymarin (100mg/kg) + PCM (2mg/kg)	73.62±3.41*	122.04±4.48*	169.8±5.4*

Statistically Significance test was done by One Way ANOVA followed by Dennett's't' test using SPSS 16.0 Windows version. *P<0.05 compared to Disease control group.

TABLE 4: EFFECTS OF PCM, SILYMARIN AND HESO ON TOTAL BILIRUBIN AND TOTAL PROTEIN OF DIFFERENT ANIMAL GROUPS

S. no.	Groups	Treatment given	Total Bilirubin Levels (mg/dl) Mean ±SEM	Total protein Levels (mg/dl) Mean ±SEM
1	Naive control	0.5% CMC (1 ml/kg)	0.68 ± 0.004	6.80 ± 0.008
2	Disease control	PCM (2mg/kg)	1.43 ± 0.003	3.88 ± 0.029
3	Test Drug dose I	HESO (200mg/kg) + PCM (2mg/kg)	0.90 ± 0.01*	5.19 ± 0.006*
4	Test drug dose II	HESO (400mg/kg) + PCM (2mg/kg)	0.76 ± 0.005*	6.11 ± 0.02*
5	Standard Drug	Silymarin (100mg/kg) + (PCM 2mg/kg)	0.71 ± 0.007*	6.74 ± 0.01*

Statistically Significance test was done by One Way ANOVA followed by Dunnett's't' test using SPSS 16.0 Windows version. *P<0.05 compared to Disease control group.

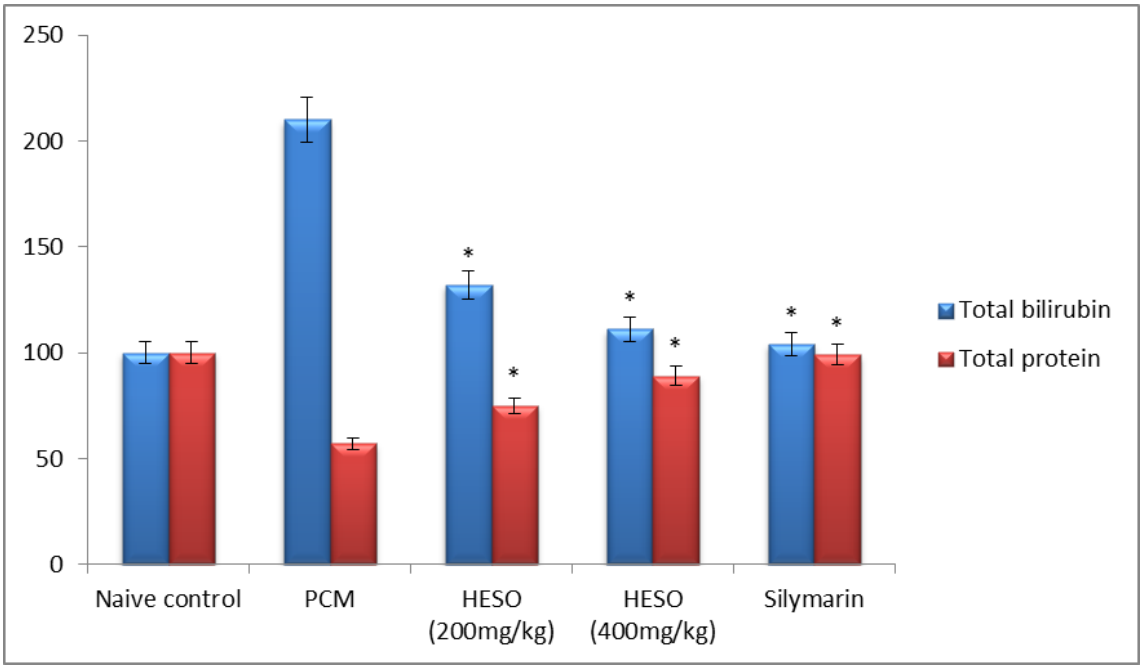


Fig. 4: Effect of PCM, SILYMARIN and HESO on Serum Bilirubin and Protein in different animal groups. Statistically Significance test was done by One Way ANOVA followed by Dunnett's't' test. *P<0.05 compared to Disease control group. Control value for Serum bilirubin- 0.68 ± 0.004, Total protein- 6.80 ± 0.008. All values are MEAN ± SEM of 6 animals per group.

4. **Tissue MDA & Serum MDA: Table 5 & fig. 5** represents that the level of lipid peroxidation (expressed as MDA) was significantly increased in the liver tissue of PCM-treated group, compared to the Control group. Pretreatment with HESO at dose of 200 and 400 mg/kg b. wt. significantly reduced PCM induced increase of TBARS content. Serum MDA was significantly increased of PCM-treated group, compared to the Control group. Pretreatment with HESO at dose of 200 and 400 mg/kg b. wt. significantly reduced PCM induced increase of serum MDA.

TABLE 5: EFFECTS OF PCM, SILYMARIN AND HESO ON TISSUE MDA & SERUM MDA OF DIFFERENT GROUPS OF ANIMALS

S. no.	Groups	Treatment given	Tissue MDA Levels (nmol/gm) Mean ±SEM	Serum MDA (10^3mM) Mean ±SEM
1	Naive control	0.5% CMC (1 ml/kg)	93.30 ± 0.12	0.22 ± 0.031
2	Disease control	PCM (2mg/kg)	295.21 ± 0.73	0.47 ± 0.062
3	Test Drug dose I	HESO (200mg/kg) + PCM (2mg/kg)	167.61 ± 0.35*	0.35 ± 0.055*
4	Test drug dose II	HESO (400mg/kg) + PCM (2mg/kg)	110.11± 0.25*	0.28 ± 0.033*
5	Standard Drug	Silymarin (100mg/kg) + PCM (2mg/kg)	102.12 ± 0.15*	0.26 ± 0.037*

Statistically Significance test was done by One Way ANOVA followed by Dunnett's 't' test using SPSS 16.0 Windows version. *P<0.05 compared to Disease control group.

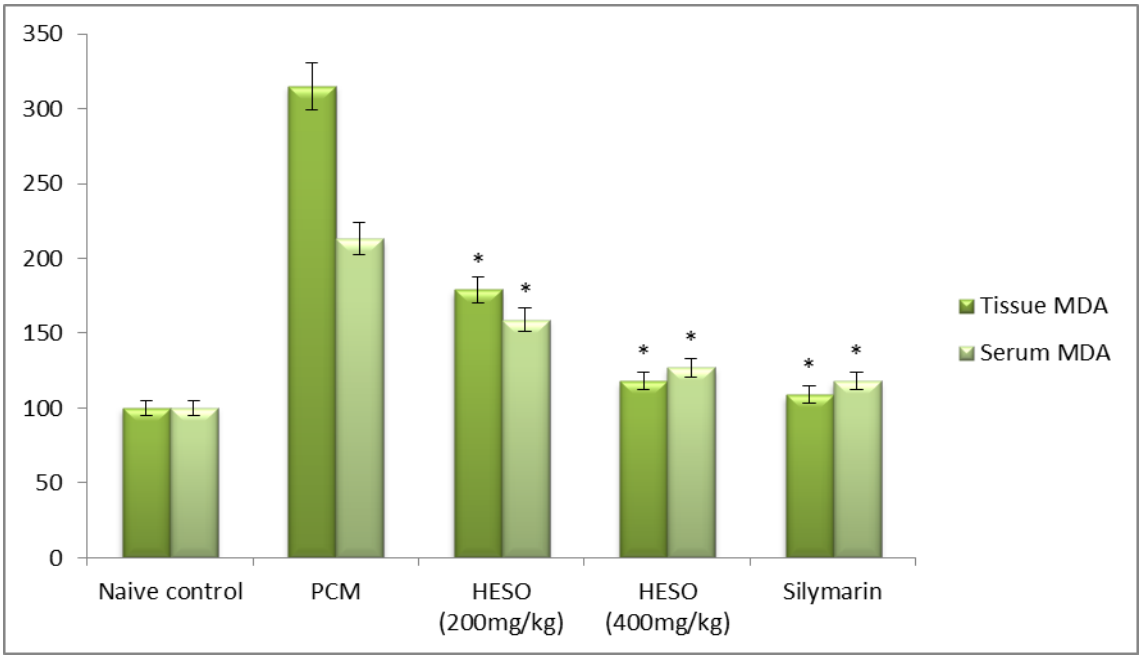


Fig 5: Effect of PCM, SILYMARIN and HESO on Tissue MDA & Serum MDA in different animal groups. Statistically Significance test was done by One Way ANOVA followed by Dunnett's 't' test. *P<0.05 compared to Disease control group. Control values for Tissue MDA- 93.30 ± 0.12, Serum MDA -0.22 ± 0.031. All values are MEAN ± SEM of 6 animals per group.

5. **TISSUE GSH, GSSG:** Table 6& fig. 6 represents that the tissue GSH levels was decreased and GSSG was increased by paracetamol treatment. The data showed that 200mg/kg and 400mg/kg of HESO and silymarin has significantly enhanced the decreased levels of tissue GSH and decreased GSSG.

TABLE 6: EFFECTS OF PCM, SILYMARIN AND HESO ON TISSUE GSH, TISSUE GSSG ON DIFFERENT ANIMAL GROUPS.

S. no.	Groups	Treatment given	Tissue GSH Levels (µg/gm) Mean ±SEM	Tissue GSSG Levels (µg/gm) Mean ±SEM
1	Naive control	0.5% CMC (1 ml/kg)	9.44 ± 0.72	0.042±0.015
2	Disease control	PCM (2mg/kg)	3.11 ± 0.18	0.085± 0.041
3	Test drug dose I	HESO (200mg/kg) + PCM (2mg/kg)	5.88 ± 0.43*	0.058± .042*
4	Test drug dose II	HESO (400mg/kg) + PCM (2mg/kg)	8.52 ± 0.69*	0.049±0.023*
5	Standard Drug	Silymarin (100mg/kg) + PCM (2mg/kg)	8.96 ± 0.71*	0.047± 0.034*

Statistically Significance test was done by One Way ANOVA followed by Dennett's't' test using SPSS 16.0 Windows version. *P<0.05 compared to Disease control group.

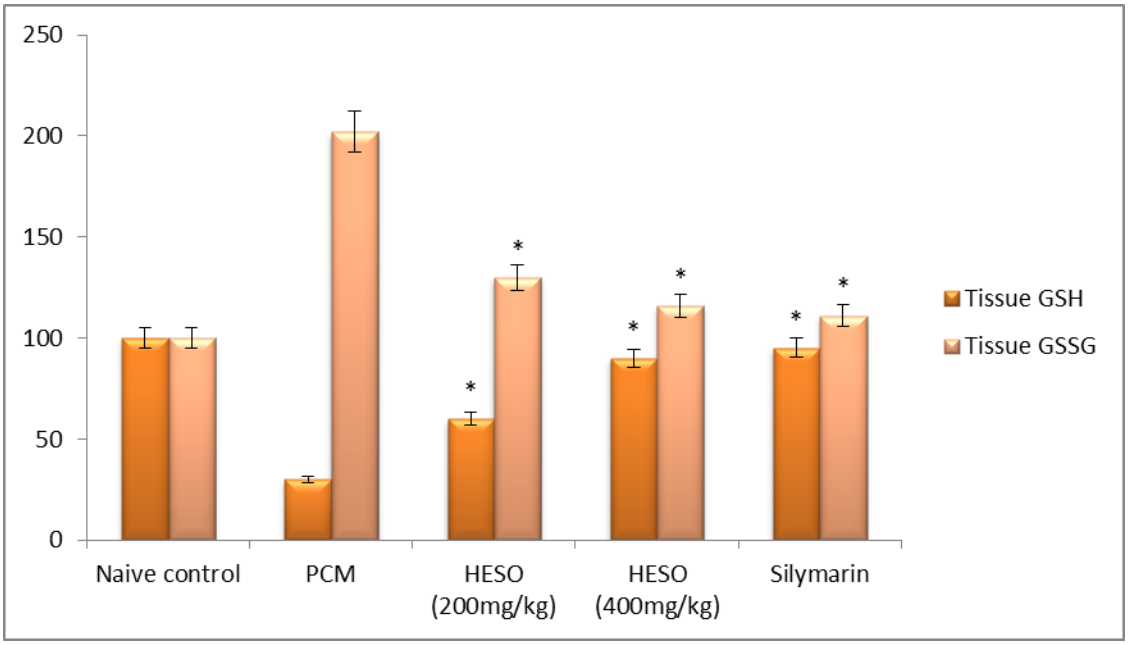
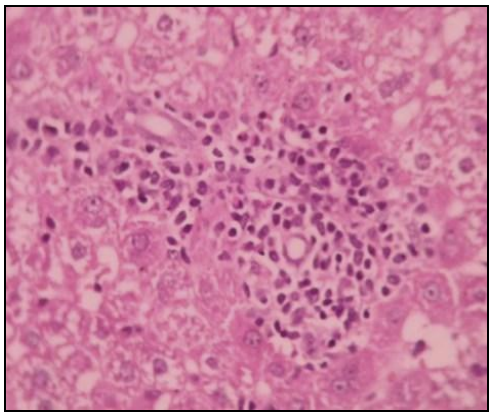
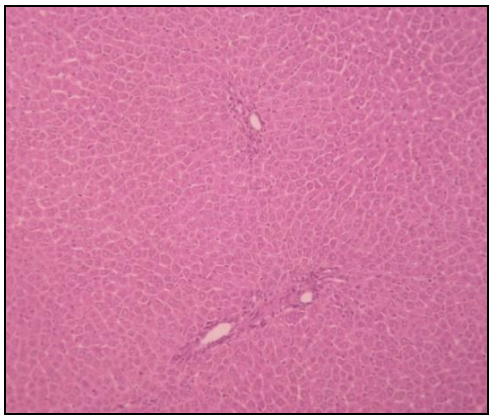


Fig 6: Effect of PCM, SILYMARIN and HESO on Tissue GSH, Tissue GSSG in different animal groups. Statistically Significance test was done by One Way ANOVA followed by Dunnett's 't' test. *P<0.05 compared to Disease control group. Control value for Tissue GSH- 9.44 ± 0.72, Tissue GSSG- 0.042 ± 0.015. All values are MEAN ± SEM of 6 animals per group.

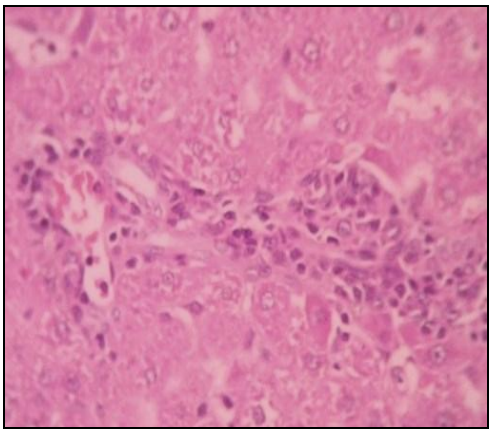
6. **HISTOLOGICAL STUDIES:** The result of histological study have presented in **fig. 7** Exposures of rat hepatocytes to paracetamol lead to histopathological changes including increased mononuclear infiltration, hemorrhage and degeneration of hepatocytes (necrosis) in comparison with blank. Liver tissue of rats treated with with HESO at dose of 200 and 400 mg/kg b. wt. and silymarin showed good recovery with absence of necrosis and fatty deposition of hepatocytes.



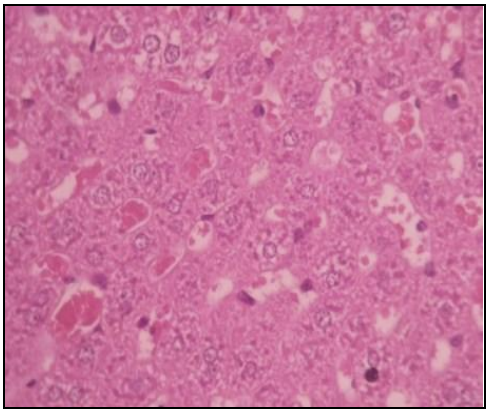
DISEASE CONTROL (PCM 2MG/KG)



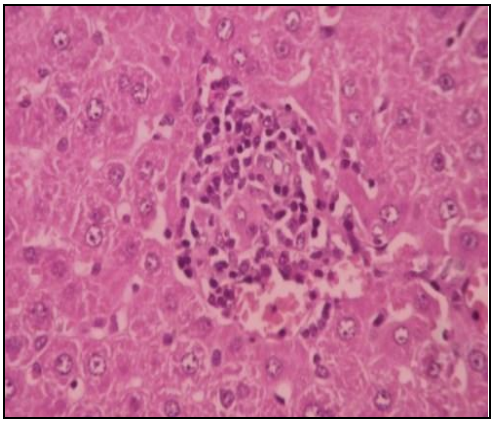
NAIVE CONTROL (5% CMC)



HESO (200MG/KG)



HESO (400MG/KG)



SILYMARIN (100MG/KG)

DISCUSSION: The main cause of hepatic injury is free radicals formation which generated during the metabolism process of xenobiotics these free radicals basically responsible for the hepatic cell injury. Liver is the main site for the metabolism of most of xenobiotics so that generation of free radical in liver is higher than other organs.

The main objective of this study is the evaluating the hepatoprotective potential of the hydro alcoholic extract of fruit. it have significant antioxidant activity ,so on the basis of this significant antioxidant activity we want to evaluate the hepatoprotective potential of the hydro alcoholic extract of *Scindapsus officinaliss* fruit in drug induced hepatotoxicity.

In this study we induce the hepatic injury to experimental animals by overdosing of drug which is called drug induced hepatotoxicity. The drugs like carbon tetrachloride, paracetamol are cause zonal necrosis to liver tissue. This is the most common type of drug-induced liver cell necrosis where the injury is largely confined to a particular zone of the liver lobule. It may manifest as a very high level of ALT and severe disturbance of liver function leading to acute liver failure.

Biochemical Parameters:

1. **Estimation of Serum Marker Enzymes:** Hepatotoxin gets converted into radicals in liver by action of enzymes & these attacks the unsaturated fatty acids of membranes in presence of oxygen to give lipid peroxides consequently. The functional integrity of hepatic mitochondria is altered, leading to liver damage. During hepatic damage, cellular enzymes like SGPT, SGOT and ALP present in the liver cells leak into the serum, resulting in increased concentrations Administration of paracetamol significantly increased all these serum enzymes. SGPT is a cytosolic enzyme primarily present in the liver.

   (a) **SGPT:** The level of SGPT in serum increases due to leakage of these cellular enzymes into plasma by toxicants induced hepatic injury. Serum levels of SGPT can increase due to damage of the tissues producing acute hepatic necrosis, such as viral hepatitis and acute cholestasis. Paracetamol induced liver damage caused due to formation of toxic metabolites leads to elevation in SGPT levels. In the current study treatment of rats with hydro acoholic extract of *Scindapsus officinalis* fruit significantly decreased the levels of SGPT in serum which is an indication of hepatoprotective activity.

   (b) **SGOT:** SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevates the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Paracetamol induced liver damage caused due to formation of toxic metabolites is associated with mild to moderate elevation of transaminases. In the current study treatment of animals with hydroalcoholic extract of *Scindapsus officinalis* fruit significantly decreased the levels of SGOT in serum which is an indicative of hepatoprotective activity.

   (c) **ALP:** In case of toxic liver, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchymal or duct cells. In the current

study treatment of animals with hydroalcoholic extract of *Scindapsus officinalis* fruit significantly decreased the levels of ALP in serum which is an an indication of hepatoprotective activity.

2. **Total Serum bilirubin:** In case of toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin. Such a situation can occur in generalized liver cell injury. Paracetamol interfere with the net uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to biliary excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease. In the current study treatment of animals with hydroalcoholic extract of *Scindapsus officinalis* fruit significantly decrease the levels of bilirubin in serum which is an indication of hepatoprotective activity.

3. **Total Protein:** Hepatocellular damage caused by antitubercular drugs decreases the total protein level in serum due to the damage to the tissues. Since the hydroalcoholic extract of *Scindapsus officinalis* fruit show increase in total protein level in serum of animals it possesses statistically significant hepatoprotective activity.

4. **Lipid Peroxidation:** Increase in the level of lipid peroxides in liver reflects the hepatocellular damage. The depletion of antioxidant defenses and/or raise in free radical production deteriorates the pro-oxidant antioxidant balance, leading to oxidative stress induced cell death. Paracetamol induced liver injury has been associated with increased amount of lipid peroxidation. Indeed, HESO extract supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that HESO extract possibly has antioxidant property to reduce paracetamol induced membrane lipid peroxidation.

5. **Tissue GSH and GSSG:** Glutathione (GSH) is a tripeptide with a gamma peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. Thiol groups are reducing agents, existing at a concentration of approximately 5 mM in animal cells. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form glutathione disulfide (GSSG), also called L(-)-Glutathione.

Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity. Glutathione exists in reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent (H++ e-) to other unstable molecules, such as reactive oxygen species. In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). Such a reaction is probable due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver). Paracetamol induced liver injury has been associated with decreased amount of GSH. Indeed, HESO extract supplementation in our study was potentially effective in preventing oxidization of GSH to GSSG suggesting that HESO extract possibly has antioxidant property to reduce paracetamol induced Hepatotoxicity.

6. **Organ to body weight indices:** OBWI is the physical parameter which indicates the ratio of the organ weight to the body weight. In case of toxic liver, wet liver weight are increased. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a fall in serum lipids in another series of experiments. It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. Treatment with hydroalcoholic extract of *Scindapsus officinalis* fruit significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant hepatoprotective activity.

Histopathological studies: In toxicant treated animals, there will be severe histopathological disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. In the present study, animals treated with extract under study exhibited minimal hepatic derangements and intact cytoarchitecture of the liver was maintained, indicating hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameters, Antioxidant parameters, and histopathological studies, it is concluded that the hydro alcoholic extract of *Scindapsus officinalis* fruit possesses hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

ACKNOWLEDGEMENT: Authors are thankful to Dr. Sudheer Bhardwaj (Director, Shriram College of Pharmacy) for providing financial support and necessary facilities for the experiment and very thankful to Mr. Manish Banker for his valuable guidance. We are also giving thanks to all supportive staff of the institute who were directly or indirectly involved in this experiment.

REFERENCES:

1. Chatterjee A, Pakrashi SC. The treatise on Indian medicinal plants. National Institute of Science Communication, New Delhi. 2001; 6.
2. Kirtikar KP, Basu BD. Indian medicinal plants, Lalit Mohan Basu, Allahabad). 1993; 3.
3. Anonymous. Indian medicinal plants; a compendium of 500 species, first edition reprint (Orient Longman Limited, Himayatnagar, Hydrabad, India), 1997;5:92-5.
4. Nandkarni KM. Indian plants and drugs with their properties and uses. Reprint Indian edition, Asiatic Publishing House. 2001.
5. Voigt JO. A catalogue of the plants. Reprint. International Book Distributors. 1984.
6. Drury CH. Ayurvedic useful plants of India. 2006.
7. Nair RV. Controversial drug plants, Universities press private limited. 2004.
8. Anonymous. The useful plants of India. Fourth reprint edition (National Intitute of Science communication, New Delhi. 2002.
9. Kirtikar KR, Basu BD. Indian medicinal plants. Second edition, III reprint, International Book distributors. 1999; 4.
10. Nandkarni AK. Indian materia medica, third revised and enlarge edition. Bombay popular Prakashan. 2002; 1.
11. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 25th edition, Nirali Prakashan. 2003.
12. Shrivastava N, Baghel RS, Baghel SS, Agrawal P, Rajput S. Gajpeepal (*Scindapsus officinalis* fruit): anatomical & pharmacological REVIEW. World Journal of Pharmacy and Pharmaceutical Sciences, 2012; 1(1):45-55.
13. Singh M, Velraj M. *In-vitro* Evaluation of *Scindapsus officinalis* (ROXB.) Schott. Fruit for Antioxidant Potential. African J. Basic and Appl. Sci., 2009; 1(3-4):83-6.
14. Friedman, SE, Grendell, JH, McQuaid, KR. Current diagnosis & treatment in gastroenterology. New York: Lang Medical Books/McGraw-Hill. 2003; 664–79.
15. Nourooz-Zadeh J, Tajaddini-Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. Elevated levels of authentic plasma hydroperoxides in NIDDM. Diabetes 1995; 44:1054-58.
16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351-8.
17. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal biochem1968; 25:192-205.

How to cite this article:
Shrivastava N, Agrawal P, Khare A, Rajput S, Baghel RS and Baghel SS: Hepatoprotective activity of *Scindapsus officinalis* fruit in Paracetamol induced Hepatotoxicity in rats. *Int J Pharm Sci Res* 2013; 4(4); 1598-1609.