International Journal of Current Trends in Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijctpr

RESEARCH ARTICLE

Evaluation of Hepatoprotective Activity of Methanolic Leaf Extract of *Evolvulus nummularius* Linn.

G. Sindhu*, V. Jayashankar Reddy, T. Usha Kiran Reddy

S V U College of Pharmaceutical Sciences, S.V University, Tirupati–517502.

ABSTRACT

The present study was aimed to explore the hepatoprotecive activity in aqueous whole plant extract of EN on rats. Acute toxicity studies were conducted in Male Wistar rats by using OECD guidelines No.423. The protective effects shown by EN with biochemical parameters (SGOT, SGPT & ALP) and histological parameters recorded with CCl_4 induced hepatotoxic rat models clearly depicts that EN possessed hepatoprotective activity. The 400 mg/kg and 200mg/kg of EN treated groups showed comparable hepatoprotective activity with standard drug sylimarin. The 400 mg/kg of methanolic extract of *Evolvulus nummularius* treated group has better action the 200 mg/kg body weight.

Keywords: *Evolvulus nummularius*, silymarin, haepatoprotective, SGOT, SGPT & ALP.

ARTICLE INFO

Corresponding Author

G. Sindhu

S V U College of Pharmaceutical Sciences,

S.V University, Tirupati–517502.A.P, India.

MS-ID: IJCTPR3786

PAPER QR-CODE

Article History: Received 19 Jan 2019, **Accepted** 27 February 2019, **Available Online**15 March 2019

Citation: *G. Sindhu, et al.* Evaluation of Hepatoprotective Activity of Methanolic Leaf Extract of *Evolvulus nummularius* Linn.. *Int. J. Curnt. Tren. Pharm, Res., Res*., 2019, 7(2): 71-77.

CONTENTS

1. Introduction

India is one of the 12 leading biodiversity centres with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. Among these, only about 7000 plants are used in Ayurveda, 600 in Siddha, 700 in unani and 30 in modern medicines (Mukherjee, 1998). During 1950-1970, approximately 100 plants based new drugs were introduced in USA drug market including deserpidine, reseinnamine, reserpine, vinblastine & vincristine which are derived from higher plants.(Harsh Mohan, 2000). Research investigations conducted on several plant

products used as liver protectives is well documented.(Vinod Kumar Kanayia, 2011) Hepatoprotective effect of some of these like *Picrorhiza kurroa, Tinospora, Cordifolia, Withania somnifera, Ricinus communis, Tephrosia purpurea,* against CCl4 and galactosamine induced hepatic injury. Some other hepatoprotective drugs are *Apium graveolens* Linn., *Boerhaavia diffusa* Linn, *Euphorbia antisyphilitica, Rubica cordifolia, Solanum lyratum, Tylophora indica, Abrus precatorius, Carica papaya, Rosmarinus tomentosus, Artemssia abrotanum* (Ausin DF, 2004).

A liver disease is always associated with cellular necrosis, increases in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. Inspite of phenomenal growth of modern medicine, there are few synthetic drugs available for the treatment of hepatic disorders. However there are several herbsor herbal formulations claimed to have possess beneficial activity in treating hepatic disorders (Achuthan, 2003).

2. Materials and Methods

Experimental Animal:

The experiment protocol described in the present study was approved by the institutional animal ethical committee (IAEC) and with the permission from committee for the purpose of control and supervision of experiments on animals (CPCSEA) ministry of social justice and empowerment, government of India (Ishak KG, 1982). Healthy adult female wistar rats weighing 150-180 Gms were used. Rats were housed in poly propylene cages, maintained under standardized condition which means that 12-hour light/dark cycle, 24±2°C, and 35 to 60% humidity and provided free access to pelleted 'sabardan' diet and purified drinking water ad libitium. The animals were not provided food for 24 hour before experimentation but allowed free access to water throughout (Hyeung Sik et al., 2008).

Plant Material:

Collection and Authentication of Plant

The plant Evolvulus nummularius was collected from in and around Tirumala hills. They were collected and authentified by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, S.V. University, Tirupati. After authentication, the leaves were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of Plant extract

The leaves were shade dried and then powdered. The powder was extracted with methanol (70%) for 72 hrs. The extract was concentrated using rotary evaporator. The extract was formulated as suspension using 1% saline as suspending agent. The % of yield of extract will be calculated and dose will be selected based on the toxicity studies (Khlifi S, 2006). Preliminary phytochemical screening (Kokate CK, 1996). Preliminary phytochemical screening of the plant extract revealed the presence of alkaloids, triterpenoids, steroids, saponins, flavonoids, tannins, phenols and absence of resins and carbohydrates (William M Lee, 1995) (Table-2).

Acute toxicity Study

The methanolic leaf extract of EN was studied for acute toxicity at a dose of 2000 mg/kg and 4000 mg/Kg p.o in wistar rats. The extract was found devoid of mortality of the animals (Kuriakase GC, 2010). So the screening doses selected for the evaluation of hepatoprotective activity as per OECD guidelines No. 423 (Annexure - 2D) fixed dose method are mentioned below.

Methanolic leaf extract of EN

1. 200 mg/kg (1/10th of 2000 mg/kg).
2. 400 mg/kg (1/10th of 4000 mg/kg).

Hepatoprotective Activity:

Experimental Design

The animals were divided into five groups of six animals each. Except the normal group all the other groups received carbon tetrachloride (CCl4) 50% v/v in liquid paraffin at a dose of 0.1 ml/kg b.w intraperitoneally for 14 days. Normal groups received normal saline orally. The standard group received silymarin 100 mg/kg orally. Test group received extract 200 mg/kg b.w and 400 mg/Kg b.w. orally. On the 14th day, blood was collected from each animal for serum analysis. The rats were sacrificed, and their livers were removed. One lobe was then fixed in 10% formalin for histopathological studies and the remaining part was subjected for antioxidant study (Mona F. Mahmond, 2012).

Table-1: Treatment schedule

S.No	Group	Treatment
1	Normal	Normal saline p.o
2	CCl_4 treated	CCl4 0.1 ml/kg b.w i.p
3	Silymarin	Ccl4 0.1 ml/kg b.w i.p + 100 mg/kg silymarin p.o
4	Test-I	Ccl40.1 ml/kg b.w i.p + 200 mg/kg p.o
5	Test-II	Ccl4 0.1 ml/kg b.w i.p + 400 mg/kg p.o

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Bonferroni test using computer based fitting program (Prism, Graph pad.). Statistical significance was determined at $P < 0.05$.

3. Results and Discussion

The results of body weight and hepatoprotective activity of methanolic extract of *Evolvulus nummularius* on CCl4 treated rats are shown in Table 3 and Table 4. The hepatic enzymes SGPT, SGOT, ALP and bilirubin in serum were significantly ($P < 0.05$) increased in CCl4 treated animals when compared to control. The methanolic extract of Evolvulus *nummularius treatments* significantly reversed the levels of SGPT, SGOT, ALP, bilirubin and ALT when compared to Ccl4 alone treated rats. Silymarin (100 mg/kg) treated animals also showed significant decrease in AST ($P<0.05$), ALT, ALP and bilirubin ($P<0.05$) levels when compared to Ccl4 alone treated rat.

Table-2: Preliminary Phytochemical Screening

Chemical Test	Inference
Alkaloids	+
Carbohydrates	
Steroids	+
Glycosides	+
Tannins	+
Resins	
Triterpenoids	+
Saponins	+
Flavonoids	+
Phenols	+

4. Conclusion

The present study demonstrated that EN (200& 400 mg/kg) exhibited significant dose dependent hepatoprotective activity against liver injury induced by CCl4. Both SGOT and SGPT and ALP levels increase due to toxic compounds affecting the integrity of liver cells. The result of the present study indicates that EN probably stabilizes the hepatic plasma membrane from Ccl4 induced damage. The protective activity of the extract may be attributed to the membrane stabilizing agents present in the EN, which may avert enzyme leakage in tissues in response to Ccl4 poisoning leading to enhanced metabolic transformations of amino acids in liver through synthesis and transformation. EN enhanced the synthesis of TP which accelerates the regeneration process and the protection of liver cells. Therefore, the increased level of proteins in serum indicates the hepatoprotective activity of EN. Antioxidant defense enzymes SOD, CAT (Giudice and Montella, 2006) and peroxidase, protect the aerobic cells against oxygen toxicity and lipid peroxidation. Both these levels were increased significantly after treatment with MEEN, which can be attributed due to the presence of a number of polyphenolics such as flavonoids (pavithra 2011). In the present investigation both the test extracts exhibited dose dependent significant reduction in biochemical marker levels and morphological parameters

Table-3: Effect of methanolic extract of leaves of EN on biochemical markers in CCl4 induced hepatotoxicity

Groups	SGPT IU/L	SGOT IU/L	ALP IU/L	TB mg/dl	TP Gm%	TC mg/dl	Triglycerides mg/dl
Normal control	23.56± 0.4167	64.52± 0.2417	134.1± 0.6079	0.7± 0.03162	6.2± 0.03162	97.44± 0.2315	85.56± 0.2315
CCl_4	57.76± 0.2786	172.6± 0.2154	280.8± 0.3311	1.74± 0.04	4.64± 0.02449	143.8± 0.3406	167.8± 0.3406
Standard silymarin 100 mg/kg	29.36± 0.3311***	84.48± 0.2577***	159.8± 0.3429***	0.9± 0.03162***	5.94± 0.05099***	102.5± 0.2332***	92.62± 0.2417***
200 mg/kg of methanolic extract of EN.	41.88± 0.3441***	145.7± 0.2871***	217.7± 0.3441***	1.56± 0.02449**	5.14± 0.02449***	128.4± 0.337***	143.6± 0.172***
400 mg/kg of methanolic extract of EN.	33.76± 0.337***	114.4± 0.3033***	174.4± 0.2482***	1.14± 0.02449***	5.72± 0.03742***	109.6± 0.2315***	113.1± 0.3611***

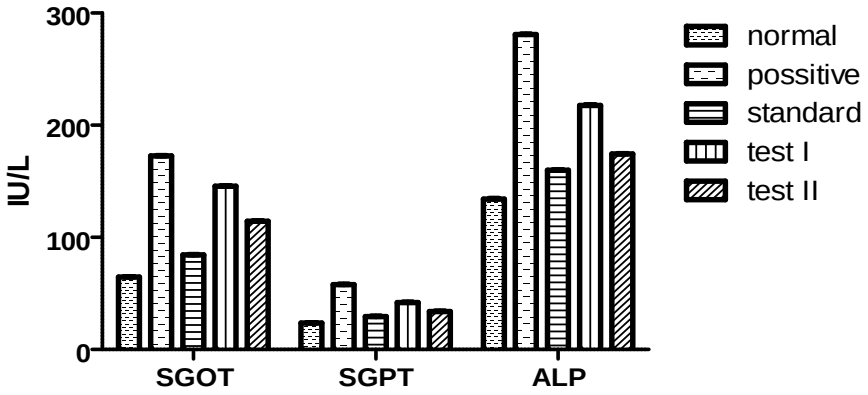
All values are shown as mean ± SEM and n=6.
*Iindicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to CCl_4 group. SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, Alk.P: Alkaline phosphatase, TB: Total bilirubin, TP: Total protein, TC: Total cholesterol.

Table-4: Effect of methanolic extract of leaves of EN on tissue parameters in CCl4 induced hepatotoxicity

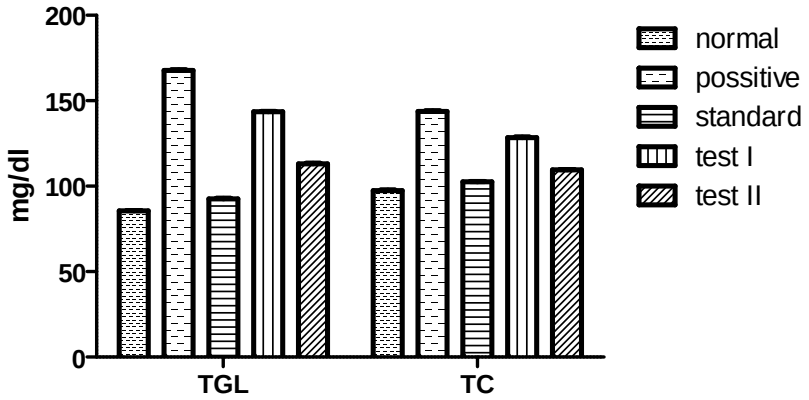
Groups	SOD U/mg proteins	CAT µM H_2O_2 consumed /mg protein	LPx µmol of MDA formed / gm tissue / hr	GSH n mol of NADPH oxidized / min mg protein
Normal control	1.906	0.402	6.32	0.28
CCl_4	0.88	0.186	9.88	0.164
Standard silymarin 100 mg/kg	1.681	0.351	7.06	0.268
200 mg/kg of	1.241	0.259	8.557	0.184

methanolic extract of EN				
400 mg/kg of methanolic extract of EN.	1.449	0.307	7.62	0.254

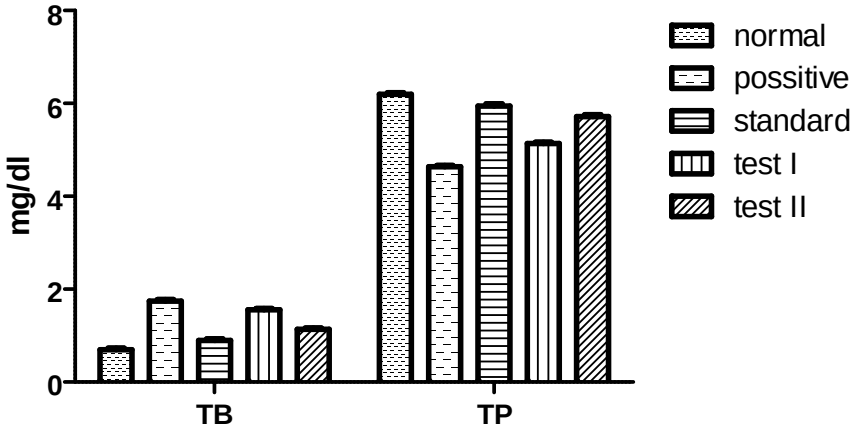
SOD (Units/mg protein)
CAT (µmol of H_2O_2 decomposed/min/mg protein)
LPx (µmol of MDA formed/gm tissue/hr)
GSH (n mol of NADPH oxidized/min/mg protein)



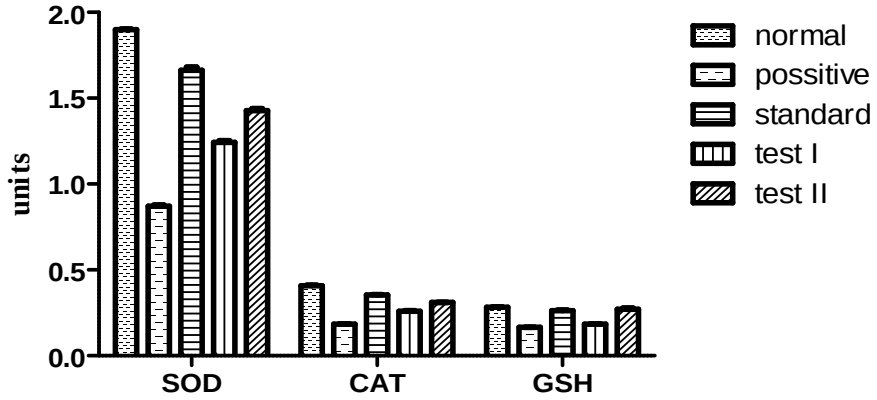
Graph-1: Effect of *Evolvulus nummularius* on Serum Biochemical Parameters in CCl_4 Induced Hepatotoxicity



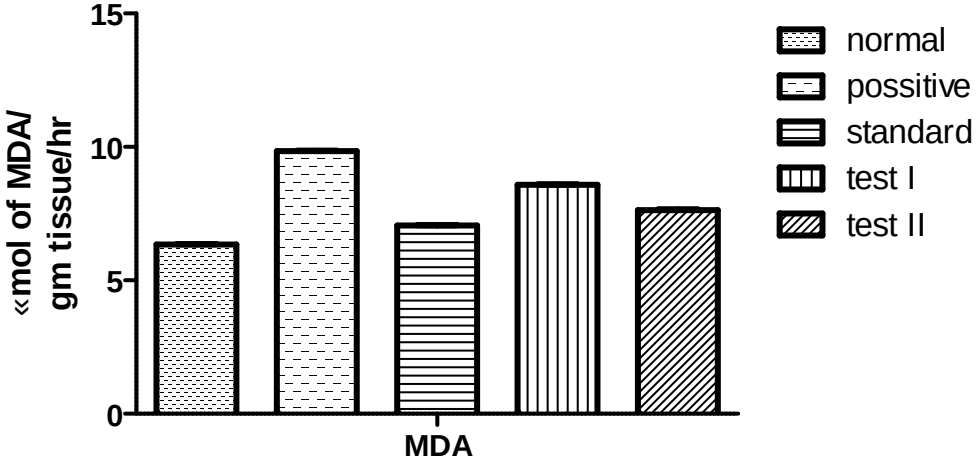
Graph-2: Effect of *Evolvulus nummularius* on serum biochemical parameters in CCl_4 induced hepatotoxicity



Graph-3: Effect of *evolvulus nummularius* on serum biochemical parameters in CCl_4 induced hepatotoxicity



Graph-4: Effect of *Evolvulus Nummularius* on Tissue Antioxidants in CCl_4 Induced Hepatotoxicity



Graph-5: Effect of *Evolvulus Nummularius* on Tissue Pro-Oxidants in Ccl_4 Induced Hepatotoxicity

Histopathological Studies:

Normal: In case of normal control (-ve control), hepatic globular structure, central vein, portal tract and kupffer cells look normal.

Suggestive: **Normal liver**. (Plate-1)

CCl_4 : In case of Ccl_4 treated group (+ve control), hepatic cells has shown extensive fatty change and ballooning of hepatocytes, more around central vein and microvasculisation fatty change. Liver sinusoids were congested.

Suggestive: **Extensive Fatty liver**. (Plate -2)

CCl_4+Silymarin:

In case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There was mild inflammatory cells and mild fatty change. There was no congestion.

Suggestive: **Regenerative changes in liver**. (Plate-3)

CCl_4+ 200mg of EN:

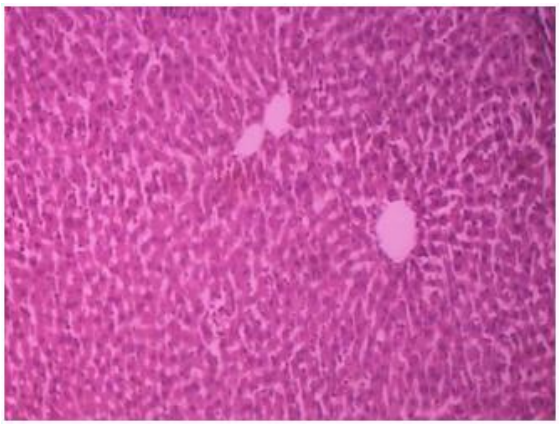
In case of with 200 mg/kg of methanolic extract of EN treated group, there was mild perportal inflammation and mild fatty change. There was mild congestion.

Suggestive: **Light regeneration of hepatocyte.** (Plate-4)

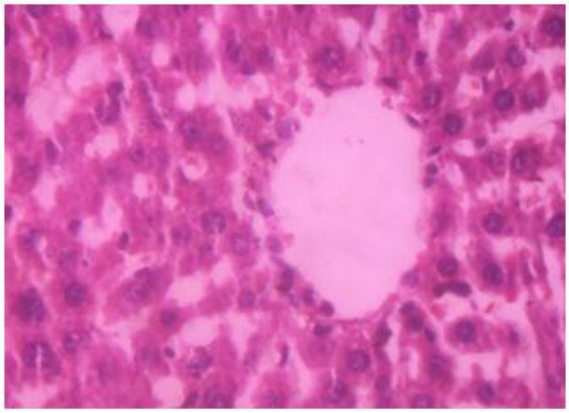
CCl_4+ 400mg of EN:

In case of with 400 mg/kg of methanolic extract of EN treated group the hepatic architecture was maintained. Central vein was congested. However, mild congestion and mild inflammation were there.

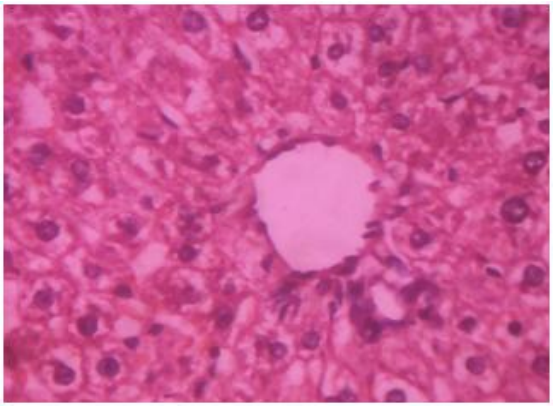
Suggestive**: Regeneration of hepatocytes.**(Plate-5)



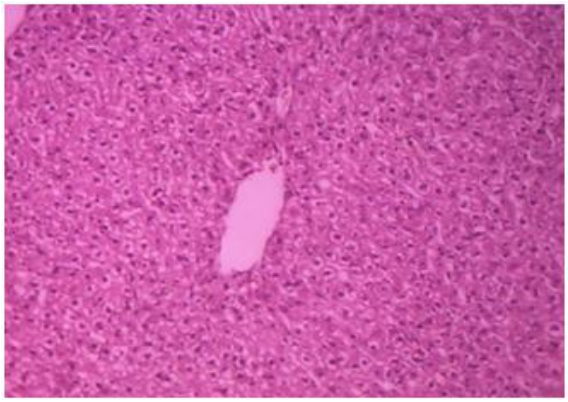
A) Normal Liver (10X)



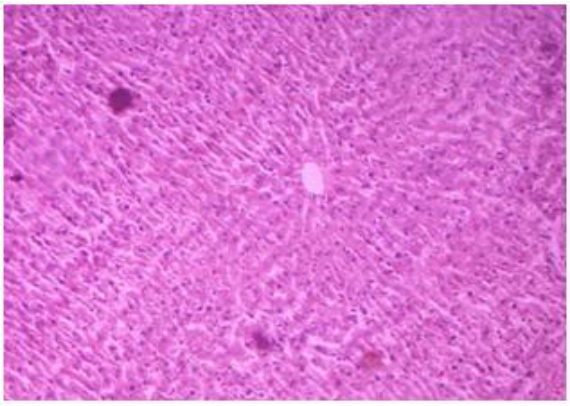
A) Normal Liver (40X)



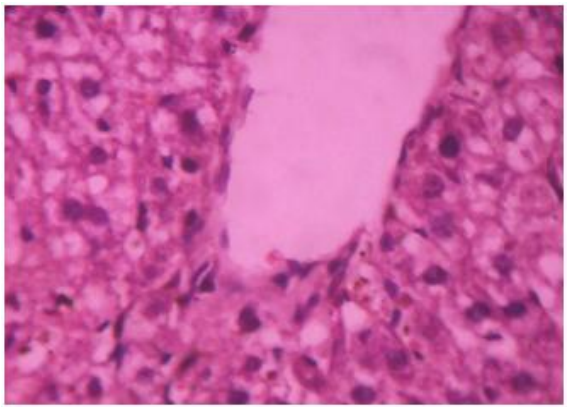
C) Silymarin + Ccl4 Liver (40X)



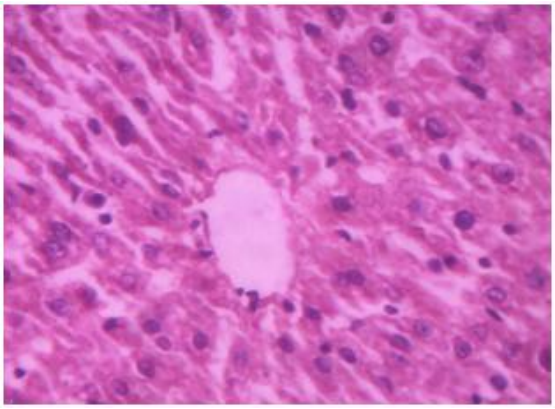
B) Ccl4 Treated Liver (10X)



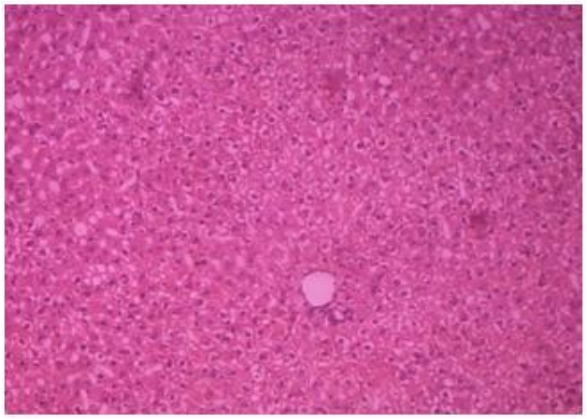
D) EN200 + Ccl4 Liver (10X)



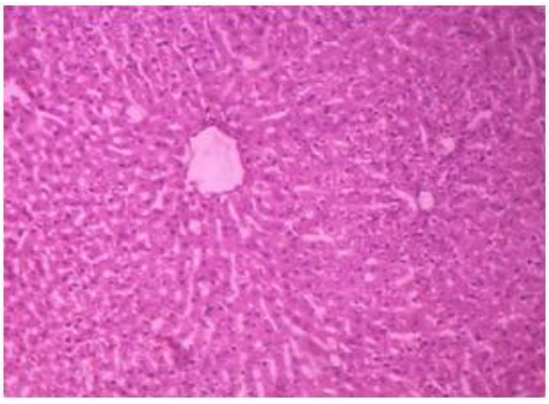
B) Ccl4 Treated Liver (40X)



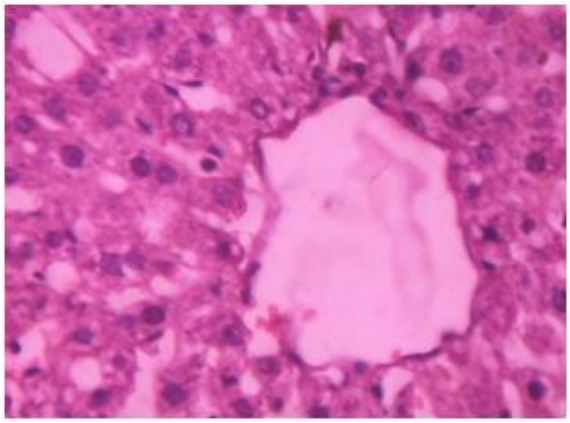
D) EN200 + Ccl4 Liver (40X)



C) Silymarin + Ccl4 Liver (10X)



E) EN400 + Ccl4 Liver (10X)



E) EN400 + Ccl_4 Liver (40X)

5. References

[1] Achuthan, C.R., B.H. Babu and J. Padikkala, 2003. Antioxidant and Hepatoprotective effects of *Rosadamascena*.Pharmaceut. Biol., 41: 357-361.

[2] Ausin DF. Florida Ethnobotany. CRC Press, New York, USA2004; 188-190.

[3] Giudice A, Montella M. (2006). Activation of the Nrf2-ARE signaling pathway: a promising strategy in cancer prevention. Bioessays, 28, 169-181.

[4] Harsh Mohan."Textbook of Pathology", Jaypee brothers Medical Publishers Ltd., New Delhi.2000; 4: 577-619.

[5] Hyeung Sik Lee, Hoeng Hui Kim, Sae Kwang Ku. Hepatoprotective effects of Artemiliae lapollaris Herba and pecorrhiza Rhizoma combinations on Ccl4 induced subacute liver damage in rats. Nutrition Research, 2008; 28 : 270-277.

[6] Ishak KG. "The liver" chapter 17 in Pathology of drug induced and toxic diseases. Riddell RH. New York: Churchill Livingstone, 1982; 459p.

[7] Khlifi S, Hachimi EI, Khalil A, Es-Safi N, Belahyan A, Tallal R, et al. In vitro antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract. Indian J Pharmacol 2006; 38(4): 276-80.

[8] Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. IV edition Pune: Nirali Prakashan, 1996.

[9] Kuriakase GC, Kurup MG. Hepatoprotective effect of Spirulinalonar on Paracetamol induced liver damage in rats, Asian J Exp. Biol Sci. 2010; 1: 614-623.

[10] Mona F. Mahmond, A. Fanmy, Marwa A. Auf. Evaluation of the hepatoprotective effect of green tea extract and selenium on Ccl4 induced fibrosis.e-SPEN Journal, 2012; 7 : 23-29

[11] Mukherjee PK, Sahu M, Suresh B. Indian Herbal Medicines. The Eastern Pharmacist, 1998; XLI (490): 21-3.

[12] Pavithra P.S., Sreevidya N, Rama S. Verma, Antibacterial and anti-oxidant activity of methanol extract of *Evolvulus nummularius*. Indian Journal of Pharmacology. 2011; 41 (5): 233-236.

[13] Vinod Kumar Kanayia, R. Irchhriya, H.K. Singh, Deepak Krilasiye, Mohini Verma, Rahul Deo Yadav, Dilip Shirhare. Evaluation of hepatoprotective activity on the leaves of Ficus benjamine Linn. J. Nat. Prod. Plant Resour., 2011; 1(3) : 59-69.

[14] William M Lee, Drug-induced Hepatotoxicity. Eng J med 1995: 26.