Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 26s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

In-Vitro Hepatoprotective Activities of Pergularia daemia and Terminalia catappa L leaf extracts

Vijaya Packiriswamy[1*], Indumathi Parameswaran[2], Vijayalakshmi Krishnamurthi[3]

[*1] Associate Professor, Department of Biochemistry, St. Peter Institute of Higher Education and Research, Avadi, Chennai - 600054.

[2] Associate Professor, Department of Biotechnology, D.G.Vaishnav College, Arumbakkam, Chennai – 600 106, Tamil Nadu, India.

[3] Associate Professor, Department of Biochemistry, Bharathi Women's College, Chennai-600108, Tamil Nadu, India.

***Corresponding author:**

Vijaya Packiriswamy

Department of Biochemistry, St. Peter Institute of Higher Education and Research(Deemed University),

Avadi, Chennai -600054.

Cite this paper as: Vijaya Packiriswamy, Indumathi Parameswaran, Vijayalakshmi Krishnamurthi, (2025) In-Vitro Hepatoprotective Activities of Pergularia daemia and Terminalia catappa L leaf extracts. *Journal of Neonatal Surgery*, 14 (26s), 1080-1089.

ABSTRACT

Plant based products are currently used for different alignments with less side effects than the chemical derived drugs. Plants are rich in secondary phytochemicals like polyphenols, flavonoids, terpenoids and tannins with lot of pharmacological activities. In this work the hydroethanolic leaf extracts of *Pergularia daemia* and *Terminalia catappa* was subjected to silica gel chromatography and gradually eluted with Hexane, Ethyl acetate, Ethanol, Methanol, Water. The column fractions were collected form each plant extracts. The above fractions were used to study the hepatoprotective activities in cell line studies. From MTT assay the Maximum cell viability was observed in two column fractions of *Pergularia daemia* and *Terminalia catappa*. These fractions are used to study the *invitro* hepatoprotective activity in different concentrations. The selected fractions were used to assessed the hepatoprotective in hepatocytes cell lines. Hepatoprotectivity was observed by cell viability and SGOT and SGPT in different concentration of extracts.

Keywords: *Hepatoprotective Activity, MTT Assy, SGPT, SGOT, Column fractions, Cell viability.*

1. INTRODUCTION

The important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds[1]. Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well[2].

In herbal medicine , whole or different plant parts like roots, bark sand leaves ,flowers, fruits and seeds are used as a herbal formulations. Crude plant material are used to sustain wellbeing and vitality for different health issues [3]. In Phytochemical, the phyto was derived from the greek, it means plants. These are naturally acquiring biological active active compounds with various health benefits and rich in micro and macronutrients[4].

Secondary metabolites are used for defense pathway, provide physical appearance like color to the plants. They are play an important role in signalling and regulation of primary metabolic pathways. Secondary metabolites are important compounds produced in other metabolic pathways, these are not essential for the functioning of plants [5]. Alkaloids, terpenes, lignins, flavonoids, curcumines and plant steroids, saponins, phenolic and glucosides are the secondary phytochemical constituents present in the plants [6].

The root extracts of *P. daemia* studies represented the presence of lots of phytochemical constituents like alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, tannins and phenolics compounds. These phytochemicals have antioxidants activity to the prevents oxidative stress and reduced degenerative diseases. The alcohol extracts of *Pergularia daemia* showed the sharp down of swelling of paw compared with standard. The reduction indicated the anti-inflammatory activity owing the presence of steroids [8].

The leaves of *Terminalia catappa* are enriched with different types of flavonoids, tannins, saponins and phytosterols. Due to phytochemical constituent these leaves and barks are used in herbal medicines for different alignments. These leaves are used as a antioxidant and anticlastogenic, liver diseases and antiplasmodium activities [9].

T. catappa leaves have the capability to prevent LPO, formation of superoxide, and their free radical scavenging activity are due the presence of tannin as a multiple antioxidant. Punicalin and punicalagin are the important phytochemicals with effective antioxidant activities [10].

2. MATERIALS AND METHODS

Collection and authentication of plant specimens

The fully mature *Pergularia daemia and Terminalia catappa* (Red leaves) leaves were collected during August – September 2012 from south Poigainallur village in Nagapattinam district of Tamilnadu, India. The *Pergularia daemia* and *Terminalia catappa* belonged to Asclepiadaceae and Combretaceae family respectively. These specimens were identified and authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science ,Plant Anatomy Research Centre, Chennai. The Voucher Specimen of *Pergularia daemia* was **PARC/2013/2118** and *Terminalia catappa* was **PARC/2014/2063.**

One kg of shade dried mature green and red leaves of *Pergularia daemia* and *Terminalia catappa* cut into small pieces and grind in to powder and used for solvent extraction. The powder was extracted with 1:1 hydro ethanol for three days in rotatory shaker at 100 rpm/min. Then the rotatory evaporator was used to distilled the solvents. The extraction steps was repeated until the pale colour produced.

Column chromatography

The Hydro alcohol extract was subjected to column chromatography using different solvent systems (Hexane, Ethyl acetate, Ethanol, Methanol and Distilled water) respectively. The fractions collected were pooled. Silica gel G (100-200 Mesh size) was used as stationary phase. The selected column fractions of TCF 2,3,4 and PDF 1,2,3 were tested for their hepatic cytotoxicity at 25,50,100,250,500 µg/ml on isolated rat hepatocytes. The selected extracts were dissolved in dimethyl sulfoxide (DMSO) (sigma, USA) and used for MTT assay.

Procedure for MTT (3-(4,5– dimethyl -2-thiazolyl)-2,5-diphenyl – tetrazolium bromide) assay.

The cytotoxicity of sample on hepatocytes cells was determined by MTT assay[11]**.** The cells (1X10 ^{5}/well) were plated in 100µl of medium /well in 96 well plates. After 48 hours of incubation the cell reaches the confluence. Then, the cells were incubated in the presence of various concentrations of plant extracts (*Pergularia daemia* and *Terminalia catappa* leaf extracts) in 0.1% DMSO for 48 hours at 37 °C. After removal of the sample solution and washed with phosphate buffered saline (pH 7.4), 20µl (0.5 mg /ml) of 0.5% (3-(4,5 – dimethyl -2-thiazolyl) -2,5-diphenyl –tetrazolium bromide (MTT) phosphate – buffered saline was added. After four hours incubation, 0.04 M HCl /Isopropanol was added. The viable cells were determined by the absorbance at 570 nm with reference at 655 nm. The absorbance were measured with a microplate reader (Bio-Rad Richman,CA), musing wells without samples as blank. All the experiments were performed in triplicate. The effect of the sample on the proliferation of rat hepatocyte cells was expressed as the % cell viability, using following formula:

% Cell viability = A_{570} of treated cells / A_{570} of control cells X 100

Isolation of rat hepatocytes

The methods developed by Sarkar and Sil.,(2006) was used for the isolation of hepatocytes with slight modifications[12]. The liver was isolated under aseptic conditions and placed in HEPES (N-2-Hydroxyethyl piperazine-N-2-ethanesulphonic acid) buffer I containing HEPES (0.01M), NaCl (0.142M), and KCl (0.0067 M), and 0.5% collagenase type IV, pH 7.6 for about 45 minutes at 37°C in an incubator with constant shaking. Hepatocytes were obtained after filtration and cold centrifugation (4°C, 200 rpm /2 minutes for 3 times) and suspended in HEPES buffer.

Primary culture of rat hepatocytes

The method of Tingstrom and obrink (1989) with slight modification was used for the primary culturing of rat hepatocytes [13]. The freshly isolated viable hepatocytes were suspended in RPMI culture medium supplemented with calf serum (10%), HEPES and gentamicin (1µg/ml). These cells (1-1.2 X10 ^{6}/ml) were then seeded into culture bottles and incubated at 37°C in atmosphere of 5% CO_2.

Preparation of rosewell park memorial institute medium (RPMI)

The powdered medium (RPMI) was dissolved in 900 ml of sterile Millipore water in an autoclaved glass conical flask under sterile condition. The antibiotics were added and stirred well with 3.7 gm of sodium carbonate until it gets dissolved completely. 10% calf serum was add and mixed well. The liquid was slowly poured into the upper portion of a media sterilization unit and filtered through a 0.2µ filter under negative pressure. The medium was immediately stored at 4 °C.

Hepatoprotective Activity

Twenty four hours after the establishment of the monolayers of hepatocytes, the medium was decanted and the culture was washed with HEPES buffer I and finally the hepatocytes were suspended in Buffer I. The hepatic cytotoxicity was induced with carbon tetrachloride (0.1N) [14]. Triplicate hepatocyte suspensions (0.1 ml) from different cultures were distributed into various culture tubes labelled as control, toxicant, standard (silymarin + toxicant) and test (test sample + toxicant). The control group received 0.1 ml of vehicle (30% DMSO) and toxicant groups received 0.1 ml of carbon tetrachloride, while the test groups received 0.1 ml of respective test solutions (25,50,100,200,250 µg/ml) followed by 0.1 ml (0.1N) of hepatotoxin. The standard groups received 0.1 ml of silymarin solution (250 µg/ml) followed by hepatotoxin. The content of the culture tubes are made up to 1 ml with HEPES buffer I. The contents of all the tubes were mixed well and incubated in a CO_2 incubator for 24 h at 37°C. In test and standard the hepatocytes were pre incubated with respective solutions for 30 min and then exposed to hepatotoxin. After incubation hepatocyte suspensions were collected to assess cell damage. Cell viability was evaluated by MTT assay. Hepatocytes suspensions were centrifuged at 200 rpm. The leakage of the enzymes GOT, GPT were determined from the supernatant.

Data analysis

The Percentage of cell viability are reported as mean ± standard deviation of three independent experiments.

3. RESULTS AND DISCUSSION

***In vitro* cytotoxicity potential of the selected leaf extracts.**

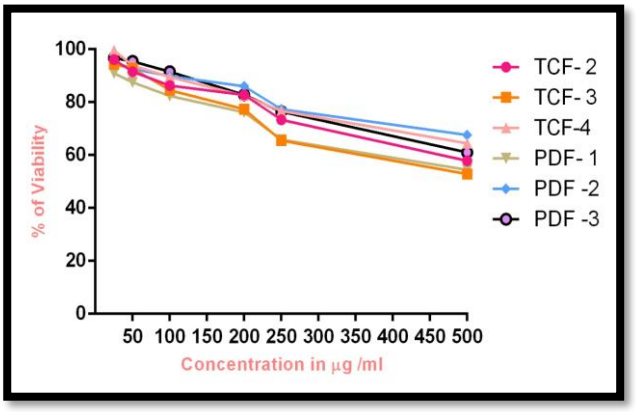
MTT assay was the most sensitive method for detecting cytotoxic events, MTT assay is mainly based on mitochondrial respiratory activity or on the enzymatic conversion of MTT in the mitochondria. It is thought that the inhibition of the mitochondrial respiration induces active oxygen related cell death. Reactive oxygen species can be generated within the mitochondria which can also damage mitochondrial components [15].

Cell cultures derived from human and animals have been used during past 30 years for determination of cytotoxicity effect caused by dental materials. Permanent cell lines and primary cells have been used as a model [16].

In the present study the cell viability of different column fraction of PD and TC leaf extracts at different concentrations (25,50,100,200,250,500 µg/ml) were represented in Figure -1. The percentage of viability of different column fractions of hydroethanolic extracts of PD (PDF - 1,2,3) and TC (TCF -2,3,4) was increased from higher to lower concentration. The rat hepatocytes experienced significant increase in cell viability in PDF2 and TCF4 than the other fractions. The % viability of PDF ranges from 95.18 ± 0.15, 93.59 ± 0.12, 89.88 ± 0.10 and 86 ± 0.18, 77.4 ± 0.03, 67.62 ± 0.05 and TCF range were 99.66 ± 0.11, 93.89 ± 0.14, 89.53 ± 0.27 and 82.46 ± 0.10, 76.7 2 ± 0.10, 64.44 ± 0.11 respectively at 25 to 500µg/ml concentration of fractions.

From MTT assay the Maximum cell viability was observed in PDF-2 and TCF- 4 column fractions of *Pergularia daemia* and *Terminalia catappa*. These fractions are used to study the invitro hepatoprotectivity.

Figure 1 : Impact of HAEPD and HAETC on cell viability



PDF – *Pergularia daemia* Fractions ,TCF – *Terminalia catappa L* Fractions

Hepatoprotective effects in freshly isolated rat hepatocyte cells

Isolated hepatocytes have been extensively used to characterize the metabolism of Xenobiotics. They offer the possibility of analyzing the pathways of metabolism in a model system under different conditions where largely maintaining the cell integrity and the intra cellular inter relationship between enzyme systems and cofactors [17].

The biochemical mechanism involved in the development of CCl_4 hepatotoxicity has long been investigated & it is now generally believed that the formation of reactive trichloromethyl radicals (CCl_3) from CCl_4 by CYP 450 is a crucial factor in the pathogenesis of CCl_4 hepatotoxicity [18]. In the presence of oxygen, CCl_3 is quickly transformed into trichloromethyl peroxy radicals (CCl_3O_2), CCl_3O_2 binds covalently to cellular proteins or lipids and initiates the lipid peroxidation in the cellular membrane [19]. This results in the leakage of cellular enzymes (LDH, GPT and GOT) and finally cellular apoptosis and necrosis [20]. Therefore, cell viability and leakage of cytosolic enzymes (LDH, GPT and GOT) have been frequently used to assess the CCl_4 hepatotoxicity [21].

In recent years, natural antioxidants, especially those in natural food or medicinal plants, draw attention of many researchers for the compounds that may be daily consumed and have many health functions. It was found in many studies that polyphenols exhibits some helpful biological effects such as antioxidative, anticarcinogenic, antihepatotoxic, antiischamic, antiallergic, antiulcerative and anti-inflammatory activities [22].

The hepatoprotective effects of hydroalcoholic extracts of *Pergularia daemia* and *Terminalia catappa* on freshly isolated rat hepatocytes intoxicated with CCl_4 are recorded in Figure-2,3,4,5. A significant increase in the levels of SGOT, SGPT ($p < 0.001$) and a significant ($p < 0.001$) reduction in the cell viability percentage were observed in hepatocytes exposed to CCl_4 when compared to normal hepatocytes **(**Figure-3).

The cells treated along with the hydroethanol extract of *Pergularia daemia* and *Terminalia catappa* showed a significant ($p < 0.001$) restoration of the altered biochemical parameters (SGOT, SGPT) towards the normal level when compared to CCl_4 treated group. The percentage viability of hepatocytes of the above extracts showed increased significantly from lower to higher concentration of column fractions when compared to CCl_4 intoxicated hepatocyte cells as shown in figure 4 and 5.

CCl_4 produces decrease in adhesive interaction of cellular surface in area of simple connection that lead to increase deformity of hepatocytes surface leading to decrease viability percentage and increase leakage of intracellular enzymes [23]. Dvorak *et al*., (2003) [24] explained the Cytotoxicity and cytoprotective were determined by assessing of cell viability, cytosolic enzymes leakage (AST, ALT, LDH, GSH) in to the culture medium.

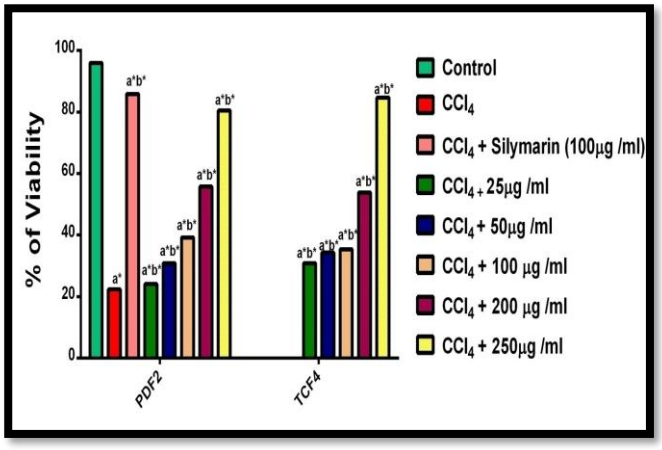
Suresh kumar S,V and Mishra S.H.,(2008) reported the hepatoprotective effect of acetone and ethanol sub fraction obtained from total ethanol extract of *Pergularia daemia* , using CCl_4-induced toxicity in primary cultured rat hepatocytes [14]. *In vitro* activity was assessed by determining the change in hepatocyte viability and other biochemical parameters such as AST, ALT and total protein. Acetone and ethanol sub fractions showed significant protective effect by restoring altered parameters in the selected *in vitro* model. The flavonoids present in acetone and ethanol sub fractions of total alcohol extract from *P. daemia* may be responsible for its significant hepatoprotective properties.

Tang XH, *et al*.,(2003) stated that the *T. catappa* inhibits the overexpression of interleukin-6 (IL-6) gene in the liver of Chemokine (C-C motif) ligand 4 (CCl_4) induced mice and the alanine aminotransferase (ALT) activity is reversed [25]. Also, histological alterations such as the infiltration of several inflammatory cells and hepatocyte swelling in injured mice are efficiently reduced by the pretreatment of *T.catappa*.

Similar pattern of results was obtained when CCl_4 intoxicated hepatocytes were treated with the silymarin. In the present study the hepatoprotective effects of Hydro ethanol extract of *Pergularia daemia* and *Terminalia catappa* was observed at 25 to 250 µg/ml concentration when compared to the standards.

In the present study the toxic effect of CCl_4 on cell membrane integrity was indicated by a significant decrease in the viability % of isolated rat hepatocytes and a significant increase in the leakage of intracellular enzymes (AST, ALT) in to culture medium. These results are in agreement with reports of Park *et al* .,(2008) [26], Xu *et al*., (2007) **[27].**

Figure 2 : Hepatoprotectivity and viability % of hepatocytes treated with PDF-2 and TCF- 4.



Values are expressed as mean ± SD.

Group – I : Control hepatocyte cells, Group – II : CCl_4 treated hepatocytes,

Group – III : CCl_4 treated hepatocytes + silymarin (250µg/ml) ,

Group – IV : CCl_4 treated hepatocytes + 25 µg/ml of PDF2,TCF4.

Group – V : CCl_4 treated hepatocytes + 50 µg/ml of PDF2,TCF4.

Group – VI : CCl_4 treated hepatocytes + 100 µg/ml of PDF2,TCF4.

Group – VII : CCl_4 treated hepatocytes + 200 µg/ml of PDF2,TCF4.

Group – VIII : CCl_4 treated hepatocytes + 250 µg/ml of PDF2,TCF4.

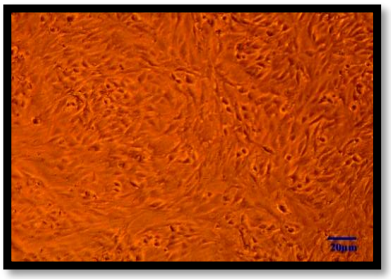
Statistical Significance : * p< 0.001,** p<0.01 and *** p<0.05, NS –Non-significant.

a - Comparison between group I with other groups.

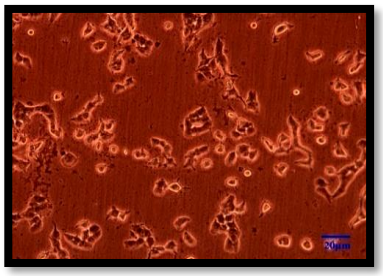
b - Comparison between group II and III, IV,V,VI,VII,VIII.

Figure 3: HEPATOPROTECTIVE POTENTIAL OF HAEPD AND HAETC ON HEPATOCYTES (Magnification 40X)

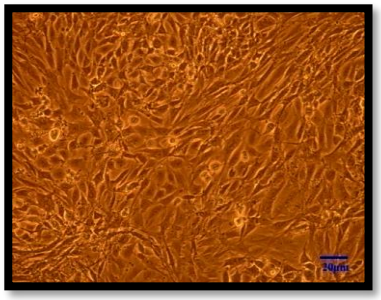
A. **Control**



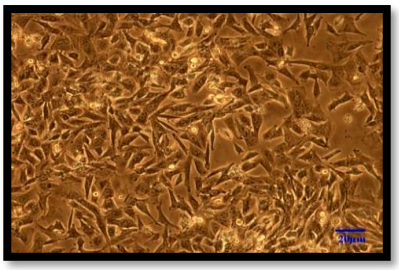
B. **.CCl_4 Treated hepatocytes**



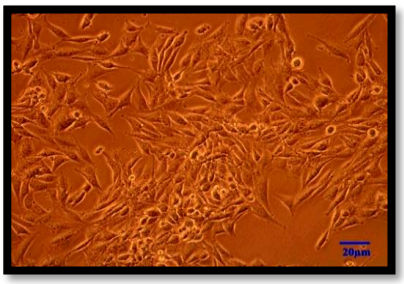
C. **CCl_4 + Silymarine**



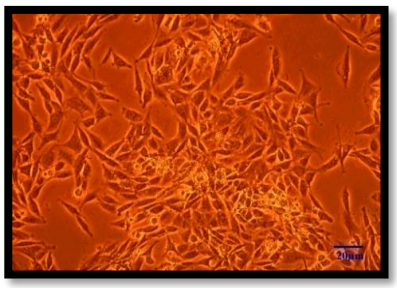
D. CCl_4 + 25 µg/ml of PDF-2



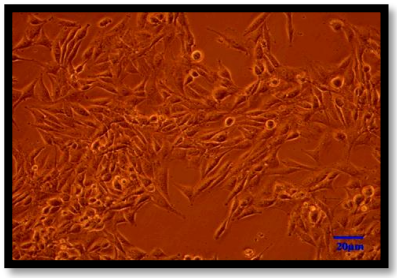
E. **CCl4 + 50 µg/ml of PDF-2**



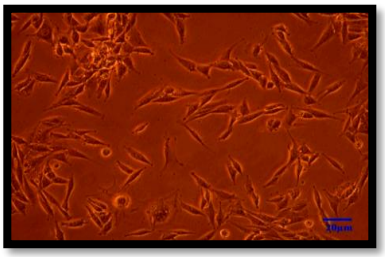
F. CCl4 + 100 µg/ml of PDF-2



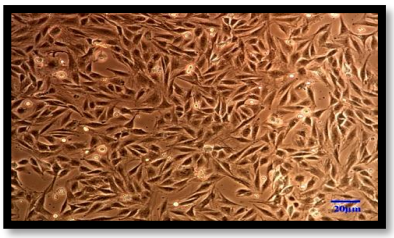
G. **CCl_4 + 200 µg/ml of PDF-2**



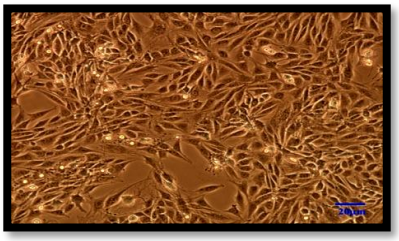
H. CCl_4 + 250 µg/ml of PDF-2



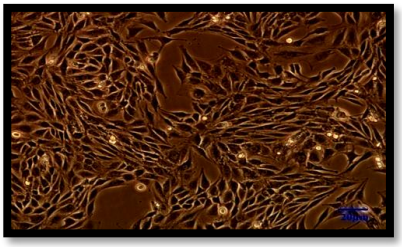
I. CCl_4 + 25 µg/ml of TCF-4



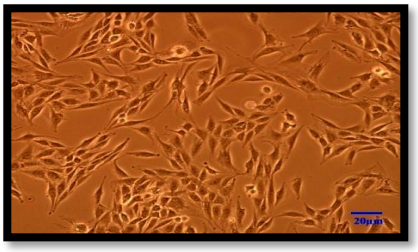
J. CCl_4 + 50 µg/ml of TCF-4



K. CCl_4 + 100 µg/ml of TCF-4



L. CCl_4 + 200 µg/ml of TCF-4



M. CCl_4 + 250 µg/ml of TCF-4

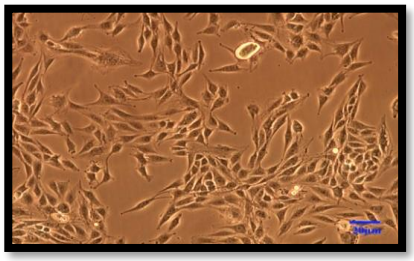
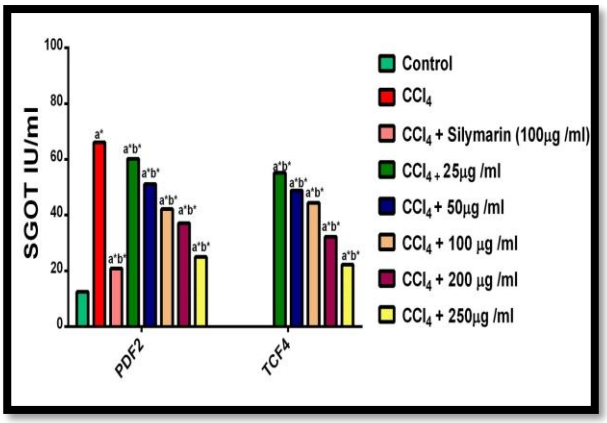
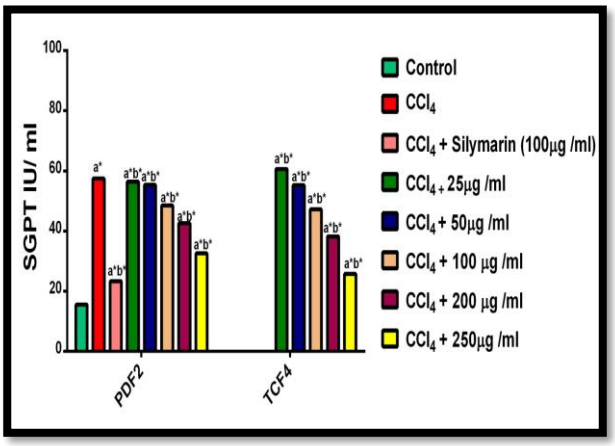


Figure 4 & 5 : Leakage of SGOT and SGPT on PDF -2 and TCF - 4 treated hepatocytes





Values are expressed as mean ± SD.

Statistical Significance : * p< 0.001,** p<0.01 and *** p<0.05, NS –Non-significant.

a - Comparison between group I with other groups .

b - Comparison between group II and III, IV, V, VI, VII, VIII.

The HAEPD and HAETC have several phytochemicals like Genistein ,α –carotene, 6-hydroxy isoflavone, 7 hydroxy 3',4'methylene dioxy isoflavone , biochanin A, apigenin -6 - Cglucoside. The peak area percentage was higher for 4',5,7 trihydroxy isoflavone (Genistein) in *Pergularia daemia* (37.46%) and *Terminalia catappa* (33.11%). Genistein posses antioxidant (Cai Q and Wei H.,1996) [28], antimicrobial (Ulanowska, *et al*., 2006) [29], anti-inflammatory (Verdrengh M, *et al* .,2003) [30] , hepatoprotective (Matsuda, *et al*.,2004) [31] , anti hypercholestermic (Suthar AC, *et al*.,2001) activities [32]. These phytoconstituents were responsible for hepatoprotective activity against CCl_4 induced rat hepatocytes. This study shows that the both the selected extract posses *in vitro* hepatoprotectivity against CCl_4 induced hepatocytes.

4. CONCLUSIONS

The major phytochemical constituents like Genistein, α –carotene, 6-hydroxy isoflavone, 7 hydroxy 3',4'methylene dioxy isoflavone, biochanin A, apigenin -6 – Cglucoside with antioxidant and anti inflammatory activities. These secondary metabolites may play an important role in reduction of CCL_4 induced liver toxicity in rat hepatocytes cells.

REFERENCES

[1] Doss A.,(2009). Preliminary phytochemical screening of some Indian medicinal plants. Anc Sci Life; 29:12-16.

[2] Pandey P, Mehta R, Upadhyay R.,(2013). Physico-chemical and preliminary phytochemical screening of *Psoralea corylifolia*. Arch Appl Sci Res; 5:261-265.

[3] Herbal Medicine. Penn State Hersney (2015). Accessedat http://pennstatehershey. adam.com/ content.aspx? productId= 107&pid=33&gid=00035 on January 12 June ,2017.

[4] Hasler CM and Blumberg JB.,(1999). Symposium on Phytochemicals: Biochemistry and Physiology. Journal

of Nutrition; 129(3): 756S-757S.

[5] Lingarao M and Savithramma N.,(2011). Phytochemical studies of *Svensonia hyderobadensis* (Walp.) Mold – A rare medicinal plant, Der Pharm. Lett, 3, 51-55.

[6] Hahn NI.,(1998). Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research. Journal of 14. the American Dietetic Association, 98: 974- 976.

[7] Bhaskar VH and Balakrishnan N.,(2009). Invitroantioxidant property of lacticiferous plants plant species from western ghats, Tamilnadu, India. Int.J.Health Res.2:163-170.

[8] Sutar NG, Sharma YP, Kendre PN, Panigrahi MK, Deshmukh TA and Jain NP., (2009). Anti-inflammatory activity of whole plant of *Pergularia daemia* linn. in Albino rats. J. Herbal Med. Toxicol., 3: 131-132.

[9] Hnawia E, Hassani L, Deharo E, Maurel S, Waikedre J, Cabalion P, Bourdy G, Valentin A, Jullian V, Fogliani B.,(2011). "Antiplasmodial activity of New Caledonia and Vanuatu traditional medicines". *Pharm Biol*;49(4):369-76.

[10] Lin CC, Hsu YF, Lin TC.,(2001). Antioxidant and free radical scavenging effects of the tannins of Terminalia catappa L. Anticancer Res .21:237-43.

[11] Mosmann TR.,(1983). "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," Journal of Immunological Methods, vol. 65, no. 1-2, pp. 55–63.

[12] Sarkar K and Sil P.C.,(2006). A 43K Da protein fromthe herb Cajanus indicus L. Protects thioacetamide induced toxicity in hepatocytes .Toxicol in Vitro 20:634 -640.

[13] Tingstrom A, Obrink B.,(1989). Distribution and dynamics of cell surface associated cell Cam 105 in cultured rat hepatocytes . Exp Cell Res 185(1):132-142.

[14] Sureshkumar S.V and Mishra S.H.,(2008). In vitro evaluation of hepatoprotective activity of Pergularia daemia Forsk. Pharmacognosy Magazine ,vol 4,16,298-302.

[15] Koizumi T., Shirakura, H., Kumagai, H., Tatsumoto, H., Suzuki, K.T.,(1996). Mechanism of cadmium-induced cytotoxicity in rat hepatocytes; cadmium-induced active oxygen related permeability changes of the plasma membrane. *Toxicology*, 114:125-134.

[16] Sabaliauskas V, Juciute R, Bukelskiene V, Rutkunas V,Vanaiene R.T, Puriene A.,(2011). In vitro evaluation of cytotoxicity of permanent prosthetic materials. Stomatologija ,Baltic Dental and Maxillofacial Journal ,13 :75- 80.

[17] Schlemper, Siegers BDJ, Paxton JW and Robertson IGC., (1993). Rat hepatocyte -mediated metabolism of the experimental anti-tumor agent N-[2'-(dimethylamino) ethyl]acridine-4-carboxamide. Xenobiotica, 13: 361–71.

[18] Ip SP and Ko KM.,(1996). The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice. A comparative study with butylated hydroxytoluene. Biochem. Pharmacol. 52:1687-1693.

[19] Levine SA and Reinhardt JH.,(1983). Biochemical-pathology initiated by free radicals, oxidant chemicals, and therapeutic drugs in the etiology of chemical hypersensitivity disease. J. Orthomol. Psychiatry, 12:166-183.

[20] Manibusan MK, Odin M, David AE .,(2007). Postulated carbon tetrachloride mode of action: a review. J. Environ. Sci. Heal. C 25:185-209.

[21] Visen P, Saraswat B, Dhawan B., (1998). Curative effect of picroliv on primary cultured rat hepatocytes against different hepatotoxins: an in vitro study. J. Pharmacol. Toxicol. Methods. 40:173-179.

[22] Shahidi F, Naczk M.,(2004). Phenolics in Food and Nutraceuticals. CRC Press LLC, Boca Raton, Florida, USA.

[23] James A, Dolak , Robert L, Waller , Eric A, Glende Jr and Recknagel , Richard O.,(2006). Liver cells calcium homeostasis in CCl4 liver injury . New Data with Fura . Journal of Biochemical Toxicology, 3 (4) :329 -342.

[24] Dvorak Z, Kosina P, Watterova D, Sinanek V, Bachled P and Ulrichova J.,(2003).Primaryculture of human hepatocytes as a tool in cytotoxicity studies : Cell protection against model toxins by flaonalignans obtained from silybum marianum. Toxicology J .,137 : 201 -212.

[25] Sureshkumar S.V and Mishra S.H.,(2008). In vitro evaluation of hepatoprotective activity of Pergularia daemia Forsk. Pharmacognosy Magazine ,vol 4,16,298-302.

[26] Tang XH, Gao J, Wang YP, Xu LZ, Zhao XN, Xu Q.,(2003). Hepatoprotective effects of chloroform extract from leaf of *Terminalia catappa* in relation to the inhibition of liver IL-6 expression.

[27] Park JY, Park CM, Kim JJ, Song YS.,(2008). Hepatoprotective activity of dandelion (*Taraxacum officinale*) water extract against D galactosamine- induced hepatitis in rats". J. Korean Soc. Food Sci. Nut. 32(2):177-183.

[28] Xu D , Wu Y, Liao Z.V, Wang H.,(2007). Protective effect of verapamil on multiple hepatotoxic factor induced liver fibrosis in rats .Pharmacol . Res., 55 (4) :280 -6.

[29] Cai Q and Wei H .,(1996) .Effect of dietary genistein on antioxidant enzyme activities in SENCAR mice. Nutrcancer , 25 (1) :1- 7.

[30] Ulanowska K, Tkaczyk A, Konopa G ,Wegrzn G.,(2006). Differential antibacterial activity of genistein arising from global inhibition of DNA,RNA and Protein synthesis in some bacterial strains. Arch Microbiol, 184 (5) :271 – 8 .

[31] Verdrengh M, Jonsson I.M ,Holmdahil R, Tarkowski A.,(2003). Genistein as an anti-inflammatory agent . Inflamm Res , 52 (8) : 341 -6.

[32] Matusda H ,Morikawa T ,Fengming X , Ninomiya K, Y oshikawa M.,(2004). New flavonoids and pterocarpane with hepatoprotective activity from the stems of Erycibe expansa. Planta Med.,70 (12): 1201 -1209.

[33] Suthar A.C, Banavalikae M .M, Biyani M.K.,(2001). Pharmacological activities of genistein ,an isoflavone from soy(Glycine max) : part II – Anticholesterol activity ,effect on osteoporosis and menopausal symptoms . Indian Journal of Experimental biology ,vol .39,pp- 520- 525.