International Journal of Ethnomedicine and Pharmacological Research

journal homepage: www.ijepr.com/journal

Hepatoprotective and antioxidative effects of aqueous extract from Karisalai Karpam siddha formulator drug on paracetamol induced injury rat

Bharathkumar G[1] And Pitchiah Kumar M[2*]

[1]Department of Siddha, The Tamil Nadu Dr. MGR Medical University, Chennai - 600 032, Tamil Nadu, India.
[2]Department of Gunapadam, Government Siddha Medical College, Chennai - 600 032, Tamil Nadu, India. Email: pitchiahkumar@yahoo.com

History of Article:

Received 12 September 2015
Received in revised from 16 September 2015
Accepted 18 September 2015
Available online 25 September 2015

ABSTRACT

Although Karisalai Karpam siddha formulator drug as a traditional medicine has been commonly used for several centuries to ameliorate some inflammatory ailments as hepatitis in India, there have been no studies of the hepatoprotective and antioxidative effects of this drug. In this paper, the hepatoprotective effect of total phenolics from Karisalai Karpam against paracetamol induced injury was examined in rat. Karisalai Karpam inhibited the cellular leakage of two enzymes, hepatocyte ASAT and ALAT, caused by these chemicals and improved cell viability. Moreover, Karisalai Karpam afforded much stronger protection than the reference drug silibinin. Meanwhile, ABTS and superoxide radicals scavenging activities of Karisalai Karpam were also determined. The present investigation is the first to report chemical-induced injury model in rat hepatocytes and provide evidence for the hepatoprotective and antioxidative effects of Karisalai Karpam. Neutralizing reactive oxygen species by nonenzymatic mechanisms may be one of main mechanisms of Karisalai Karpam against chemical-induced hepatocyte injury. Furthermore, the total phenolic content of Karisalai Karpam and its main component type were quantified. These data support the folkloric uses of Karisalai Karpam in the treatment of hepatitis and other hepatic related diseases.

Keywords: Karisalai Karpam; Hepatoprotective; Antioxidative; Toxicity

INTRODUCTION

The satisfying welfares of traditional Siddha medicines have been accepted for centuries. Although there is still lack of proof for clarification of their typical mechanisms, unlike with Western medicine, it is still widely believed by people from East Asia and beginning to be established by the rest of the world. Karisalai Karpam, as a folk medicine for the treatment of some ailments associated with inflammation as hepatitis for over three hundred years, is widely distributed in tamilnadu, especially in south India. Paracetamol is a common antipyretic agent which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses (Mitchell *et al.*, 1973). Paracetamol is mainly metabolised in liver to excretable glucuronide and sulphate conjugates (Wong et al.,

1981). However, hepatoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 (Savides and Oehme, 1983) to a highly reactive metabolite *N*-acetyl-*p*-benzoquinoneimine (Vermeulen, *et al.,* 1992). Due to liver injury, the transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in the serum. Protection against paracetamol induced toxicity has been used as a test for a potential hepatoprotective agent by several investigators (Jafri *et al.,* 1999)

The pharmacological action of crude drug is determined by the nature of its constituents. Thus, the plant species can be considered as a contributor for definite physiological effects from a huge number of compounds for example alkaloids, terpenoids, flavonoids, glycosides and phenolics. These chemical compounds are mostly responsible for the desired beneficial properties as plants are known as a source of secondary metabolites that come with a variety of structural arrangements and properties with interesting biological activities (De Fatima *et al.,* 2002). Therefore, plants continue to be major source of medicine, as they have throughout human history (Prince and Prabakaran, 2011). However, the pharmacological studies of Karisalai Karpam have rarely been reported. It is the only report that the aqueous extract from this plant exhibited antiviral activity. It is healthy known that hepatocyte injury is one of pathological changes of hepatitis. Oxidative stress is considered to play a prominent causative role in many diseases including liver damage (Kiso et al., 1984). Now that Karisalai Karpam as a folk medicine was widely used to treat hepatic disorders. Therefore, it is possible that the plant possesses hepatoprotective and antioxidant effects. To test this hypothesis, based on our previous researches on phytochemistry of this plant (Bharathkumar and Pitchiah Kumar, 2014) and the potent anti-inflammatory activity of Karisalai Karpam. We initiate a program aiming at validating the hepatoprotective and antioxidant activities of TPLP.

MATERIAL AND METHODS

Karisalai Karpam formulated using *Karisalankanni* (*Eclipta prostrata* L. 15%), *Mañjal karisalai* (*Wedelia calendulaceae* L. 15%), *Avuri* (*Indigofera tinctoria* L. 15%), *Kottakkarandai* (*Sphaeranthus indicus* L. 15%), *Vallarai* (*Centella asiatica* L. 15%), *Kuppaimeni* (*Acalypha indica* L. 15%), *Siruseruppadai* (*Coldenia procumbens* L. 5%), juice of *E. prostrata* L. and *W. calendulaceae* L. (q.s.).

2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay

ABTS radical scavenging activity of aqueous extract from Karisalai Karpam was determined according to Re *et al.* 1999. ABTS radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 hrs in the dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of vitamin C. Similarly, in the test Group 1 ml reaction mixture comprised of 950 µl of ABTS solution and 50 µl of the extract solutions. The reaction mixture was vortexed for 10 seconds and after 6 minutes absorbance was recorded at 734 nm against distilled water using an ELICO (SL150) ultraviolet-visible (UV-Vis) spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

Superoxide radical scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971) in the presence of the riboflavin-light-NBT system, as described earlier Tripathi and Pandey 1999; Tripathi *et al.* 1998. Each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM ethylenediaminetetraacetic acid, NBT (75 µM) and different concentration of different solvent extract of Karisalai Karpam sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 minute at 560 nm using an ELICO (SL150) UV-Vis spectrophotometer. Identical tubes with the reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

% Super oxide radical scavenging capacity= $([A_0-A_1]/A_0) \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of organic solvent extract or standard.

Inhibition of lipid peroxidation

Lipid peroxidation induced by Fe2+-ascarbate system in egg yolk by the method of Bishayee and Balasubramanian 1971, was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.* 1979. The reaction mixture contained egg yolk 0.1 ml (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO4 (NH4)2SO4.7H2O (0.06 mM); and various concentrations of different solvent extracts of Karisalai Karpam in a final volume of 0.5 ml. The reaction mixture was incubated at 37°C for 1 hr. After the incubation period, 0.4 ml was removed and treated with 0.2 ml sodium dodecyl sulfate (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100°C for 1 hr. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The butanol-pyridine layer was removed, and its absorbance at 532 nm (ELICO [SL150] UV-Vis spectrophotometer) was measured to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density of treatments with that of the control. Ascorbic acid was used as a standard.

Inhibition of lipid peroxidation (%) by the extract was calculated according to 1-(E/C) × 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample

$(Abs_{532}+TBA-Abs_{532}-TBA)$.

Nitric oxide radical scavenging activity

Nitric radical scavenging capacity of different solvent extracts of Karisalai Karpam was measured according to the method described by Olabinri *et al.* 2010. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of ethanolic extract and incubated at room temperature for 150 minute. After incubation period, 0.2 ml of griess reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546 nm against blank. All readings were taken in triplicate and curcumin was used as a standard. The % inhibition was calculated by following equation.

% Nitric oxide radical scavenging capacity=([A0-A1]/A0) ×100

Where A0 was the absorbance of control and A1 was the absorbance of different solvent extract.

Animals

Healthy adult Wistar albino rat weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air conditioning. A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^{o} C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India. **Approval reference number-SU/CLATR/IAEC/IV/013/2016.**

Induction and Treatment on Paracetamol induced hepatotoxicity

Paracetamol induced hepatotoxicity model was adopted for the study method as described by Aiyalu and Muthusamy, 2012. The rats were divided into 5 groups of 6 animals each. Animal belongs to group I (Control) administered with normal saline 5 ml/kg (p.o). No treatment given to animals belongs to group II (Disease control) from 0 -7 days, from 8th to 14thday, paracetamol suspension was given orally at the dose of 2 g/kg body weight. Animals belongs to group III (Vehicle control) pretreated with honey, 40mg/kg, p.o and then administered with paracetamol suspension (2gm/kg,bw),p.o from day 8 to 14 along with honey. Animals belongs to group IV and V pretreated with low (200mg/kg) and high dose (400mg/kg) of study drug Karisalai Karpamin honey, p.o and then administered with paracetamol suspension (2gm/kg,bw),p.o from day 8 to 14 along with test drug. At the end of the experimental period, the rats were fasted overnight and sacrificed. Blood and liver samples were collected for biochemical and histological studies.

Biochemical investigations

At the end of the experimental rats were fastened overnight and sacrificed after the last day treatment and blood was collected in vacutainer tubes. Tubes were centrifuged at 4,000 RPM for 20 mints. Serum was separated from blood, stored in

eppendhrof tubes at -4°C and labeled[3]. Serum samples of control and treated rats were analyzed for biochemical investigations including Asparate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), direct bilirubin (DB), indirect bilirubin (IB) and total bilirubin (TB) were determined using Mindray BS-120 autoanalyser by standard methods using enzyme assay kits (Mindray Medical International Limited, India).

Histopathological evaluation

Appearances of organs were observed and the index of each organ was calculated. These organs included of kidney, liver, brain, heart, lung, spleen, stomach. Histological slides of organs were made and observed under the microscope. The pathological observations of these tissues were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

Masson's trichrome staining

5µm thick sections of liver samples were prepared for Masson's trichrome (Sigma, USA) staining as a marker for detecting the degree of fibrosis and observing the collagen fibers developed in liver tissues. Examination of the slides was performed under a light microscope, and digital images were captured using Olympus CX 41 microscope at the magnification ×40.

Masson's trichrome stained liver showing fibrous tissue. The fibrous tissue is stained blue while the cytoplasm of hepatocytes are stained red. The nuclei can be seen as dark red to black structures within cells; Collagen is the fibrous tissue are stained Blue (with aniline blue).

Statistical analysis

The statistical analysis will be carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error .A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

RESULT AND DISCUSSION

ABTS radical activity

Aqueous extract of Karisalai Karpam exhibited a powerful scavenging activity for ABTS radical cations in a concentration dependent manner (Table-1), showing a direct role in catching free radicals. Maximum inhibition was observed with the aqueous extract ranges from 29.14±2.13 to 74.23±0.91 at 5-20 µl/ml of Karisalai Karpam. This property may be credited to the presence of polyphenolics and flavones in the Chooranam of Karisalai Karpam. ABTS radical involves a hydrogen atom transfer process (Huk *et al.,* 1998). In this assay, the good antioxidant activity on ABTS radical of Karisalai Karpam may be attributed to a direct role in trapping free radicals by donating hydrogen atom.

Table-1. ABTS radical activity of Karisalai Karpam

Different concentration of Karisalai Karpam	[a] Percentage of radical activity with different solvent extracts of Karisalai Karpam on ABTS	
	Aqueous extract	Vitamin-C
5µl/ml	29.14±2.13	23.93±0.72
10µl/ml	41.1±1.9	36.74±2.34
15µl/ml	57.36±0.93	54.59±1.86
20µl/ml	74.23±0.91	73.37±2.97

All the observations in different groups showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a] Mean ± SD.

Inhibition of lipid peroxidation

Aqueous extract of Karisalai Karpam also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed with total aqueous decoction 24 to 81% than Vitamin C 21 to 80% at 20 µg/ml respectively than other fractions (Table-2). This embarrassment of lipid peroxidation possibly either due to chelation of Fe or by corner of the free radicals. Iron also is playing a major role for the formation of lipid peroxidation in the body. Lipid peroxidation is one of the main manifestations of oxidative damage and it has been found to play an important role in the toxicity and carcinogenicity. Several reports indicated that exposure to certain food items increased Lipid peroxidation in liver. As it is known that lipid peroxidation is the net result of any free radical attack on membrane and other lipid constituents present in the system, the lipid peroxidation may

be enzymatic(Fe/NADPH) or non-enzymatic (Fe/ascorbic acid) (Aboul-Enein *et al.,* 2003).

Table-2. Inhibition of lipid peroxidation induced by $FeSO_4$ using egg yolk homogenates as lipid rich media

Different concentration of Karisalai Karpam	[a] Inhibition Percentage of lipid peroxidation	
	Aqueous extract	Vitamin-C
5µl/ml	24±2.49	21±1.5
10µl/ml	43±0.71	39±0.52
15µl/ml	62±1.69	59±1.55
20µl/ml	81±2.05	80±1.80

There was a significant ($^{**}P < 0.05$ and $^{*}P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

Superoxide anion scavenging activity

Superoxide radicals by photochemical decrease of nitro blue tetrazolium (NBT) in the occurrence of a riboflavin-light-NBT system, which is one of the standard methods. The total fractions aqueous of Karisalai Karpam exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner (Table-3). The aqueous decoction fraction had highest Superoxide radicals scavenging percentage 79.50±0.79 at 20 µg/ml and the Vitamin C. Removal of superoxide in a concentration dependent manner by any solvent fractions may be attributed to the direct reaction of its phytomolecules with inhibition of the enzymes. Numerous biological reactions generate superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide anion radical are potential precursors of damaging oxygen species and thus the study of the scavenging of this radical is important (Pietta, 2000).

Nitric oxide scavenging assay

Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane. Inhibition increased with increasing concentration of the extract. Present study with five different solvent extracts of Karisalai Karpam showed Nitric oxide scavenging activity. The good result observed that aqueous decoction with scavenging ranges 81.04±1.67 at 20 µg/ml than vitamin C compared with 76.29±0.85 20 µg/ml for vitamin C which served as positive control (Tale-4).

Table-3. Superoxide anion scavenging activity

Different concentration of Karisalai Karpam	[a] Inhibition Percentage of lipid peroxidation	
	Aqueous extract	Vitamin-C
5µl/ml	25.08±0.79	19.12±0.98
10µl/ml	42.75±0.82	41.56±2.74
15µl/ml	61.13±1.04	60.33±1.79
20µl/ml	79.50±0.95	78.48±3.01

The present study demonstrated five different solvent acts as Nitric oxide scavenging due to extracts contain poly phenol compounds; free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage (Zou and Lu, 2004).

Table-4. Nitric oxide scavenging activity

Different concentration of Karisalai Karpam	[a] Percentage of Nitric oxide scavenging	
	Aqueous extract	Vitamin-C
5µl/ml	30.28±1.07	28.56±1.89
10µl/ml	45.07±1.08	42.32±1.33
15µl/ml	63.84±0.19	60.81±2.06
20µl/ml	81.45±0.97	76.29±0.85

[a] All the observations in different extract showed significant ($P < 0.01$) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). [a]Mean ± SD.

Effect of karisalai karpam on serum liver enzyme level in paracetamol induced hepatotoxicity

The result of the study clearly shows that rats treated with paracetamol (2gm/kg) belongs to group II shown significant elevation in the level of AST, ALT, ALP, total, direct and indirect bilirubin when compare to group I saline treated rats (Table-5). Similarly rats treated with honey 40mg/kg belongs to group III has shown very mild decrease in the level of such markers. Treatment with Karisalai Karpam at the dose of 200 and 400 mg/kg to group IV and V showed significant decrease in the level of AST, ALT, ALP, total, direct and indirect bilirubin. Paracetamol is reported to produce acute toxic effect at high doses which leads to liver damage as a result of its

bioactivation by the cytochrome P450 system to a toxic electrophile, N-acetyl p-benzoquinone imine (NAPQI), which covalently binds to tissue macromolecules, probably oxidizes lipids or the critical sulfhydryl groups (protein thiols) and alters the homeostasis of calcium (Haorah et al., 2008; Manzo-Avalos and Saavedra-Molina, 2010; Mansouri et al., 2001).

Table-5. Effect of Karisalai Karpam on Serum Liver enzyme level in Paracetamol induced hepatotoxicity

Group	Treatment	(AST) (IU/ml)	(ALT) (IU/L)	(ALP) (IU/L)	Serum Bilirubin		
					Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Indirect Bilirubin mg/dl
Group I	Normal saline 5 ml/kg	69.33±2.91	26.67±2.5	105.2±5.1	0.64±0.06	0.24±0.03	0.39±0.04
Group II	Paracetamol 2gm/kg	110±6.2	60.67±1.28	145±4.3	2.21±0.08	0.85±0.03	1.36±0.05
Group III	Paracetamol 2gm/kg + Honey 40mg/kg	99.3±3.18	51.5±1.58	136.3±2.45	1.78±0.09	0.71±0.04	1.07±0.06
Group IV	Paracetamol 2gm/kg + KK 200 mg/kg	89.3±2.4	44.67±1.3	129.2±2.38	1.26±0.04	0.50±0.01	0.76±0.02
Group V	Paracetamol 2gm/kg + KK 400 mg/kg	81.33±1.8	37±2.59	121.7±1.2	0.86±0.05	0.32±0.02	0.54±0.03

Values are expressed as mean ± S.E.M. (*N*=6). Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001. One way ANOVA followed by Dunnett's test.

Effect of karisalai karpam on liver weight of rats in paracetamol induced hepatotoxicity

Liver weights of rats treated with paracetamol (2gm/kg) were significantly increased when compared to that of the control group treated with saline. Similarly rats treated with honey 40mg/kg belongs to group III has shown marked decrease in liver weight. Treatment with Karisalai Karpam at the dose of 200 and 400 mg/kg to group IV and V showed significant decrease in the liver weight (Table-6).

Table-6. Effect of Karisalai Karpam on Liver weight of rats in Paracetamol induced hepatotoxicity

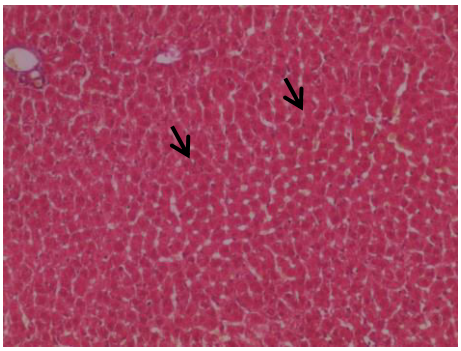
Group	Treatment	Weight of the Liver in grams
Group I	Normal saline 5 ml/kg	5.28±0.20
Group II	Paracetamol 2gm/kg	9.33±0.18*
Group III	Paracetamol 2gm/kg + Honey 40mg/kg	8.18±0.19*
Group IV	Paracetamol 2gm/kg + KK 200 mg/kg	7.55±0.15*
Group V	Paracetamol 2gm/kg + KK 400 mg/kg	6.58±0.13*

Values are expressed as mean ± S.E.M. (*N*=6). Symbols represent statistical significance: *p<0.05, **p<0.01, ***p<0.001. One way ANOVA followed by Dunnett's test.

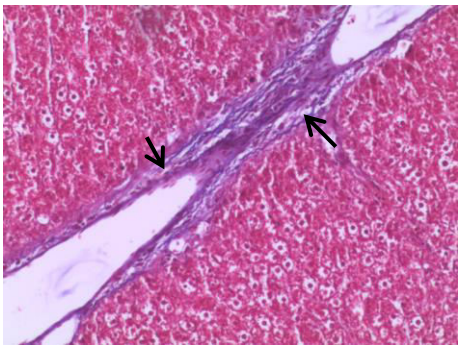
Masson's trichrome staining of paracetamol induced liver injury in rat

Normal hepatocytes with integrated cytoplasm was observed in liver sections of control group rats further no signs of fibrous tissue in or around the central and portal vein. Microscopic observation of liver sample belongs to paracetamol treated group revealed significantly increased level of collagen deposition around the central vein indicating severe fibrosis. Sample belongs to group III shown higher level of fibrous tissue formation in the peri portal areas with diffused pattern of collagen deposition. The degree of fibrosis and collagen deposition was greatly reduced in trichrome stained liver of group IV, V treated with low (200mg/kg) and high dose (400mg/kg) of Karisalai Karpam (Fig-1).

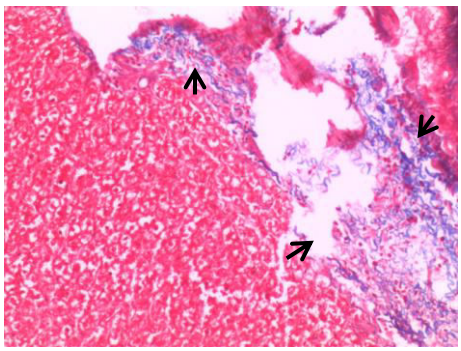
Fig-1. Masson's trichrome staining of Paracetamol Induced liver injury in Rats Histopathology of Liver



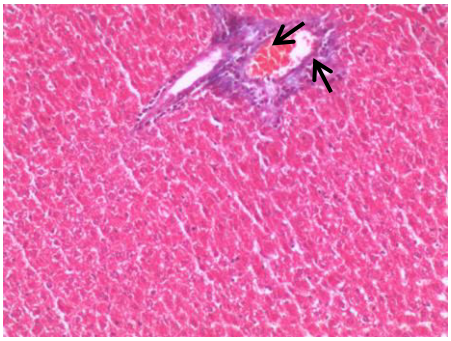
Group-I



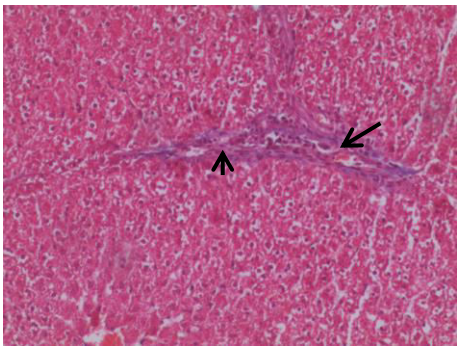
Group-III



Group-II



Group-IV



Group-V

REFERENCE

1. Aboul-Enein AM, El Baz FK, El-Baroty GS, Youssef AM, Abd El-Baky HH (2003). Antioxidant activity of algal extracts on lipid peroxidation. J. Med. Sci. 3: 87-98.

2. Aiyalu R and Muthusamy P. (2012). Hepatoprotective effect of ethanolic extract of *Trichosantheslobata*on paracetamol-induced liver toxicity in rats. Chinese Medicine. 7: pp. 12:1-6.

3. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971;44(1):276-87.

4. Bharathkumar and Pitchiah Kumar (2014). A study on phytochemical properties and antioxidant activity of different solvent extract of siddha drug karisalai karpam chooranam in *in vitro* condition Asian J Pharm Clin Res, Vol 7, Issue 5: 250-254.

5. Bishayee S, Balasubramanian AS. Lipid peroxide formation in rat brain. J Neurochem 1971;18(6):909-20.

6. De Fatima, A.B.M., Oleiveira, R.L.B., Mendes, S.S., Silva, P.D.A., Antoniolli, A.R., Vilar, J.C., Cavalcanti, S.C.D.H., Blank, A.F., 2002. Seed germination, phenology and antiedematogenic activity of Peperomiapellucida. Pharmacology (2): pp.12.

7. Haorah J, Ramirez S.H, Floreani N, Gorantla S, Morsey B, Persidsky Y. (2008). Mechanism of alcohol-induced oxidative stress and neuronal injury, FreeRadic. Biol. Med. 45 (11): pp. 1542–1550.

8. Huk I, Brovkovych V, Nanobash VJ, (1998). Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. Br J Surg. 85: pp.1080–5.

9. Kiso, Y., Tohkin, M., Hikino, H., Hattori, M., Sakamoto, T., Namba, T., 1984. Mechanism of antihepatotoxic activity of glycerrhizin. I. Effect of free radical generation and lipid peroxidation. Planta Medica 50: 298–302.

10. Mansouri A, Demeilliers C, Amsellem S, Pessayre D, Fromenty B. (2001). Acuteethanol administration oxidatively damages and depletes mitochondrial DNAin mouse liver, brain, heart, and skeletal muscles: protective effects ofantioxidants, J. Pharm. Exp. Ther. 298 (2) 737–743.

11. Manzo-Avalos S and Saavedra-Molina A. (2010). Cellular and mitochondrial effects ofalcohol consumption, Int. J. Environ. Res. Public Health 7 (12) 4281–4304.

12. Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillette, J.R., Brodie, B.N., 1973. Acetaminophan induced hepatic necrosis. 1. Role of drug metabolism. J. Pharmacol. Exp. Ther. 187, 185–194.

13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95(2):351-8.

14. Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. *In vitro* evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. Res J Biol Sci 2010;5(1):102-5.

15. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63:1035-42.

16. Prince L., Prabakaran P., 2011. Pelagia research library. Asian J. Plant Sci. Res. 1 (1): pp.84-87.

17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26(9-10):1231-7.

18. Savides, M.C., Oehme, F.W., 1983. Acetaminophen and its toxicity. J. Appl. Toxicol. 3, 95–111.

19. Tondon, V and Gupta RK. (2005). Effect of Vitex negundo on oxidativestress. Indian Journal of Pharmacology. pp. 37: 37–45.

20. Tripathi YB, Pandey E. Role of alcoholic extract of shoot of *Hypericum perforatum* Linn on lipid peroxidation and various species of free radicals in rats. Indian J Exp Biol 1999;37(6):567-71.

21. Tripathi YB, Sharma M, Upadhyay BN, Kumar DS. Antioxidant properties of *Rubia cordifoli*. Br J Phytotherapy 1998;4(4):163-7.

22. Vermeulen, N.P.E., Bessems, J.G.M., Van de Streat, R., 1992. Molecular aspects of paracetamol induced hepatotoxicity and its mechanism based prevention. Drug Metab. Rev. 24, 367–407.

23. Wong, S., Wong, M.M., Seligmann, O., Wagner, H., 1988. Drugs for liver therapy part 16. Anthraquinone glycoside from seeds of *C. tora*. Phytochemistry 28 (1), 211–214.

24. Zou YP, Lu YH. Antioxidant activity of a flavonoids rich extract of *Hypericum perforatum* L. *in vitro*. J Agric Food Chem 2004;52:5032-9.