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HEPATOPROTECTIVE EFFECT OF *PERGULARIA DAEMIA* AND *TERMINALIA CATAPPA* L. LEAF EXTRACTS ON CCl_4 INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

Hydroalcoholic extracts of *Pergularia daemia (HAEPD)* and *Terminalia catappa* L.(HAETC) were evaluated for the hepatoprotective and antioxidant activities in albino rats. The plant extracts (200mg/Kg, p.o.) showed remarkable hepatoprotective and antioxidant activity against CCl_4 induced hepatotoxic rats. The healthy wistar albino rats were grouped into seven with six rats in each group. Group I: Untreated rat kept as a control. Group II: Treated with 30% of CCl_4 (1ml/kg ,b.wt, ip).Group III: The Rats treated with standard drug (Silymarin-25mg/kg ,b.wt, po) followed by CCl_4. Group IV,V : The Rats pre treated with HAEPD and HAETC (200mg/kg,b.wt,po) followed by 30% CCl_4 . Group VI,VII: The rats first received 30% CCl_4 and post treated with HAEPD and HAETC (200mg/kg,b.wt,po) for the period of twenty five days. The CCl_4 induced rats showed a significant increase in SGOT, SGPT, alkaline phosphatase(ALK), ACP, LDH,LPO activity and with a reduction of SOD, catalase, glutathione peroxidase(GPX) and glutathione -S-transferase(GST) activity. The rats treated with two different plant extracts (200mg/Kg) has significantly ($P<0.001$) altered the activities of serum marker enzymes and antioxidant levels near to normal. The Histopathological changes of liver samples were compared with respective control. The results indicate the hepatoprotective and antioxidant properties of *Pergularia daemia* (HAEPD) and *Terminalia catappa* L. (HAETC) against CCl_4 induced hepatotoxicity in rats.

KEYWORDS: *Pergularia daemia*, *Terminalia catappa*, Hepatoprotective, Antioxidant, Histopathology, CCl_4.

INTRODUCTION

Liver plays an important role in metabolism, detoxification and excretion of many xenobiotic compounds. Because of its anatomical location and its great capacity for xenobiotic metabolism, it is frequently the target for toxic chemicals. Although viral infection is one of the main causes of liver injury, xenobiotics, excessive drug therapy, environmental pollutants and chronic alcohol ingestion can also cause hepatic injury. Cancer & chemotherapeutic drugs cause liver toxicity and it has been widely reported.[1] Most of these toxic chemicals have been reported to generate free radicals and reactive oxygen species which are the major culprits in liver pathogenesis.[2]

Recently, the most common *in-vivo* model used in the investigation of new hepatoprotective agents has been a well-characterized rodent model of liver injury induced by carbon tetrachloride (CCl_4), a chemical hepatotoxin that causes free radical-mediated hepatocellular damage.[3] CCl_4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl_4 by Cytochrome P_{450} to the trichloromethyl radicals, which lead to membrane lipid peroxidation and finally to cell necrosis. [4] The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of pro inflammatory mediators.[5]

Since time immemorial, mankind has made the use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agents.[6]

Pergularia daemia (Forsk.) Chiov. (Asclepiadaceae) is a foetid smelling laticiferous twiner found in the plains throughout the hot parts of India, ascending to an altitude of 1000 m in the Himalayas. *Pergularia daemia* is known as “Veliparuthi” in Tamil, “Uttaravaruni” in Sanskrit and “Utranajutuka” in Hindi.[7] In different folk and Ayurvedic system of medicine

the plant has been documented for antifertility,[8] wound healing,[9] antidiabetic,[10] hepatoprotective,[11] cardiovascular effect,[12] antibacterial activity.[13]

Terminalia catappa belongs to the family Combretaceae also known as Indian almond is a large, spreading tree distributed throughout the tropics in coastal environments in India. The dried leaves are used for fish pathogen treatment, as a substitute to antibiotics. The leaves have antioxidant as well as anti clastogenic properties.[14] The leaves of the plant contain flavonoids in rich quantity,these flavonoids are responsible for anti-ulcer activity. The various extracts of leaves and bark of *T. catappa* have been reported to be anticancer, anti-HIV.[15] and hepato-protective,[16] as well as anti-inflammatory.[17] antihepatitis,[18] anti diabetic.[19] and aphrodisiac activities.[20] The present study is aimed to evaluate the hepatoprotective activity of hydro alcohol extracts of the leaves of *Pergularia daemia* and *Terminalia catappa* L. against CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS

(i) Collection of Plant Sample

The fully matured leaves *of Pergularia daemia* & *Terminalia catappa* (Red leaves) were collected in August – September 2012 from South Poigainallur Village in Nagapattinam district of Tamilnadu, India and the Plant was taxonomically identified by Dr.P.Jayaraman, Plant Anatomy Research Centre, West Tambaram ,Chennai.The Voucher Specimen of *Pergularia daemia (PARC/2013/2118).)Terminalia catappa was (PARC/2014/2063).*

(ii) Preparation of extract

Matured leaves *of Pergularia daemia* &*Terminalia catappa* (Red leaves) were shade dried at room temperature and were powdered by using mechanical grinder. The hydro alcohol extracts of *Pergularia daemia* &*Terminalia catappa* were obtained by soxhlet apparatus & extracting at 65°C till discoloration. The extract was kept in air tight vials after complete evaporation and was stored in refrigerator till use.

(iii) Experimental Animals &Design

Healthy Wistar strain of male rats, two to three months old and weighing 150-200gms were purchased from King Institute, Guindy, Chennai, Tamil Nadu, India. The animals were maintained at Saveetha University, Chennai, Tamil Nadu, India. (Ethical clearance No:SU/BRULAC/RD/012/2014). The animals were allowed to acclimatize to laboratory conditions for a period of 5 days prior to the experiment. Six animals were housed per cage,

so as to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard conditions of 12-hrs light/dark cycle and at an ambient temperature at 23±2°C, with 65±5% humidity. Animals were fed with standard pelleted diet and given free access to water *ad libitum*. All the studies were conducted according to the ethical guidance of CPCSEA after obtaining necessary clearance from the Ethical committee.

The animals were divided into seven groups (each of 6 rats) as follows: **Control (Group I**): Controls received olive oil (vehicle) (1 ml/kg b.wt,p.o). at every 72 hrs for 10 days.**CCl_4 Intoxicated (GroupII)**: Received 30% CCl_4(1ml/Kg,b.wt,i.p)at every 72 hrs for 10 days. **Standard (GroupIII)**: Received Silymarin (25mg/kg, b.wt,p.o)for 15 days and 30% CCl_4(1ml/Kg, b.wt ,i.p) at every 72 hrs for 10 days. **Pre treatment(GroupIV)**: Received hydroalcohol extract of *Pergularia daemia* (200 mg/kg,b.wt,p.o.) for first 15 days and followed by 30% CCl_4(1ml/Kg,b.wt,i.p)at every 72 hrs for next10 days. (**Group V)** : Received hydroalcohol extract of *Terminalia catappa* (200 mg/kg,b.wt,p.o.) for first 15 days and followed by 30% CCl_4(1ml/Kg,b.wt,i.p)at every 72 hrs for next10 days. **Post treatment(Group VI)**: Received 30% CCl_4(1ml/Kg,b.wt,i.p)at every 72 hrs for first 10 days and followed by (200 mg/kg, b.wt ,p.o.)hydroalcohol extract of *Pergularia daemia* for next 15 days.(**Group VII)** : Received 30% CCl_4(1ml/Kg, b.wt i.p.) at every 72 hrs for first 10 days and followed by (200 mg/kg, b.wt,p.o.)hydroalcohol extract of *Terminalia catappa* for next 15 days.

(iv) Collection of Blood

At the end of the 25 th day of experimental period ,all the animals were sacrificed by cervical decapitation .The blood was collected and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 minutes and analyzed for various biochemical parameters.

(v) Assessment of liver function

Separated serum samples were used for determination of SGOT & SGPT[21] ,ALK[22] , LDH[23] & ACP[24] were assayed by the standard methods.

(vi) Assessment of antioxidant properties

The levels of lipid peroxides,[25] Activity of Superoxide dismutase (SOD).[26] and catalase (CAT). [27] activities were estimated in liver tissue. Glutathione peroxidase (GPx) activity

was measured.[28] in the liver tissue with modifications.[29] The total reduced glutathione (GSH),[30] (GST). [31] were estimated in liver tissue.

(vii) Histopathological studies

Animals were sacrificed to remove the liver. The liver was fixed immediately in 10% neutral buffered formalin ,dehydrated in different grades of alcohol, cleared in xylene, embedded in paraffin wax, sectioned at 4-6 µ thick & stained with Haematoxylin & Eosin[32] and examined microscopically.

(viii) Statistical Analysis

Statistical analysis was done by using SPSS 20 version and graph pad prism 6 software. All results were presented as mean value ± standard deviation (SD) for six samples in each group. One-way analysis of variance (ANOVA) with post hoc multiple comparison test was used for statistical analysis of collected data. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of HAEPD and HAETC on serum transaminase., alkaline phosphatase levels in CCl_4 induced liver damage in animals are summarized in **Table(1) and fig-1**. In CCl_4 treated group there was a significant($p<0.001$) elevation of hepatospecific serum markers SGOT,SGPT ALP,ACP, LDH when compared to the control group. On pre and post treatment with HAEPD and HAETC & Silymarin the activities of the above enzymes were found to reach normal level.

Table 1: Effect of hydroethanol extracts of *Pergularia daemia (*HAEPD) *and Terminalia catappa (*HAETC) *on liver marker enzymes in the serum of control and experimental animals.*

Groups	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	LDH(IU/L)	ACP(KAU)
Group – I	81.06± 0.93	48.25 ± 0.22	153.25 ± 0.07	423.18±0.39	4.11±0.01
Group – II	283.33 ± 18.61^{a*}	110 ± 1.41 ^{a*}	320 ± 3.40 ^{a*}	566.50±0.14 ^{a*}	14.70±0.14 ^{a*}
Group – III	181.66 ± 9.3 ^{a*b*}	70.30 ± 0.66 ^{a*b*}	165.33 ± 1.86 ^{a*b*}	436.40±0.14 ^{a*b*}	6.15±0.01 ^{a*b*}
Pre treatment					
Group – IV	210 ± 7.07 ^{a*b*}	89 ± 1.41 ^{a*b*}	181 ± 1.41 ^{a*b*}	506.72±0.01 ^{a*b*}	10.50±0.14 ^{a*b*}
Group – V	200 ± 1.41 ^{a*b*}	68 ±1.41 ^{a*b*}	178 ± 1.41 ^{a*b*}	506.14±0.01 ^{a*b*}	10.70±0.14 ^{a*b*}
Post treatment					
Group –VI	190 ± 1.41 ^{a*b*}	61.80 ± 0.63 ^{a*b*}	176 ± 1.41 ^{a*b*}	491.10±0.01 ^{a*b*}	8.13±0.01 ^{a*b*}
Group –VII	195 ± 1.41 ^{a*b*}	78 ± 1.41 ^{a*b*}	170 ± 1.41 ^{a*b*}	454.45±0.01 ^{a*b*}	9.30±0.14 ^{a*b*}

Values are expressed as mean ± SD, n=6.Group – I - Control rats, Group – II – CCl_4 Intoxicated animals, Group – III – Co-treated with silymarin(25mg/kg b.w) &CCl_4 ,Group – IV Pre treated with HAEPD (200mg/kg b.w),Group-V Pre treated with HAETC (200mg/kg b.w),Group–VIPost treated with HAEPD(200mg/kg b.w),Group VII Post treated with HAETC (200mg/kg b.w).Statistical Significance : * p< 0.001,** p<0.01 and *** p<0.05,NS –Non-significant. a - as compared with group I &II ' b - as compared with group II&III,IV,V,VI,VII.

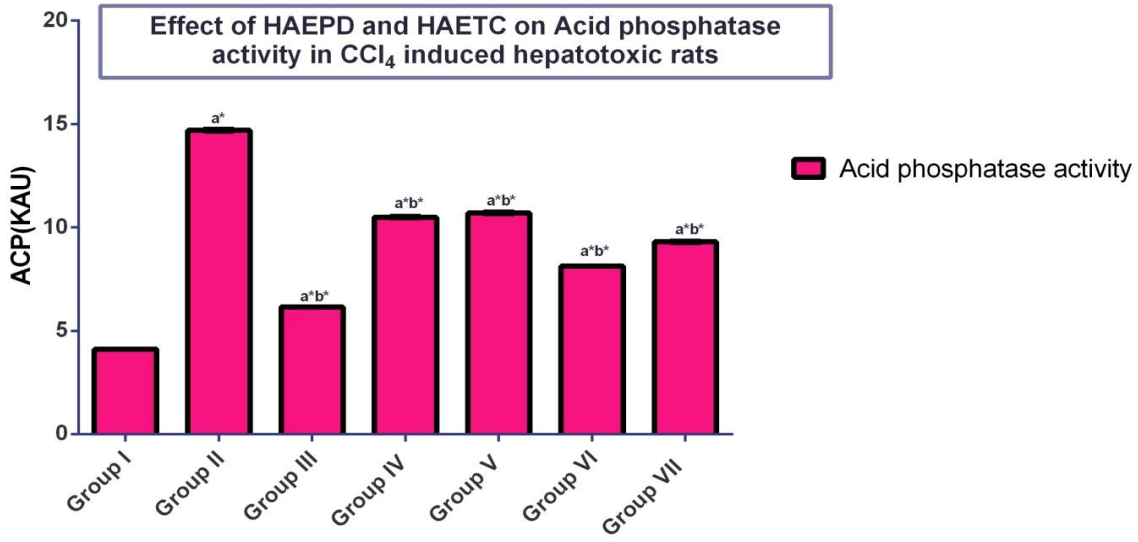

Fig 1: Effect of extracts on ACP in experimental animals

Table 2: Effect of hydroethanol extracts of *Pergularia daemia (*HAEPD) *and Terminalia catappa(*HAETC) *on Antioxidants in liver tissue.*

Group	Nonenzymatic antioxidant	enzymatic antioxidants			
	GSH(µg/gm)	GPX (µm/min/mg)	CAT (µm/mg/min)	SOD(U/gm)	GST (µm/min/mg)
Group – I	43.70± 0.77	3.98±0.30	61.5±0.34	5.70±0.14	6.50±0.14
Group – II	30 ± 1.41^{a*}	1.67±0.13 ^{a*}	45.3±0.22 ^{a*}	4.10±0.03 ^{a*}	3.20±0.01 ^{a*}
Group – III	37.5 ± 1^{a*b*}	3.73±0.03 ^{a**b*}	58.52±0.32 ^{a*b*}	5.22±0.02 ^{a*b*}	4..23±0.01 ^{a*b*}
Group – IV	32.5±0.70^{a*b*}	3.05±0.03 ^{a*b*}	48.58±0.13 ^{a*b*}	4.72±0.02 ^{a*b*}	3.55±0.01 ^{a*b*}
Group – V	34.3±1.27^{a*b*}	2.75±0.10 ^{a*b*}	49±1.41^{a*} ^{b*}	4.57± 0.04 ^{a*b*}	3.91±0.01 ^{a*b*}
Group –VI	35.8±0.59^{a*b*}	3.20±0.13^{a*b*}	52.17±0.02^{a*b*}	5.10±0.03 ^{a*b*}	3.89±0.01 ^{a*b*}
Group –VII	37.3±0.22^{a*b*}	3.58±0.13 ^{a*b*}	54.10±0.07 ^{a*b*}	5.02±0.01 ^{a*b*}	4.32±0.01 ^{a*b*}

Values are expressed as mean ± SD, n=6.Group – I - Control rats, Group – II – CCl_4 Intoxicated animals, Group – III – Co-treated with silymarin(25mg/kg b.w) &CCl_4 ,Group – IV:Pre treated with HAEPD (200mg/kg b.w),Group -V: Pre treated with HAETC (200mg/kg

b.w),Group–VI:Post treated with HAEPD(200mg/kg b.w),Group VII:Post treated with HAETC (200mg/kg b.w) Statistical Significance : * p< 0.001,** p<0.01 and *** p<0.05,NS –Non-significant. a - as compared with group I &II , b - as compared with group II&III,IV,V,VI,VII.

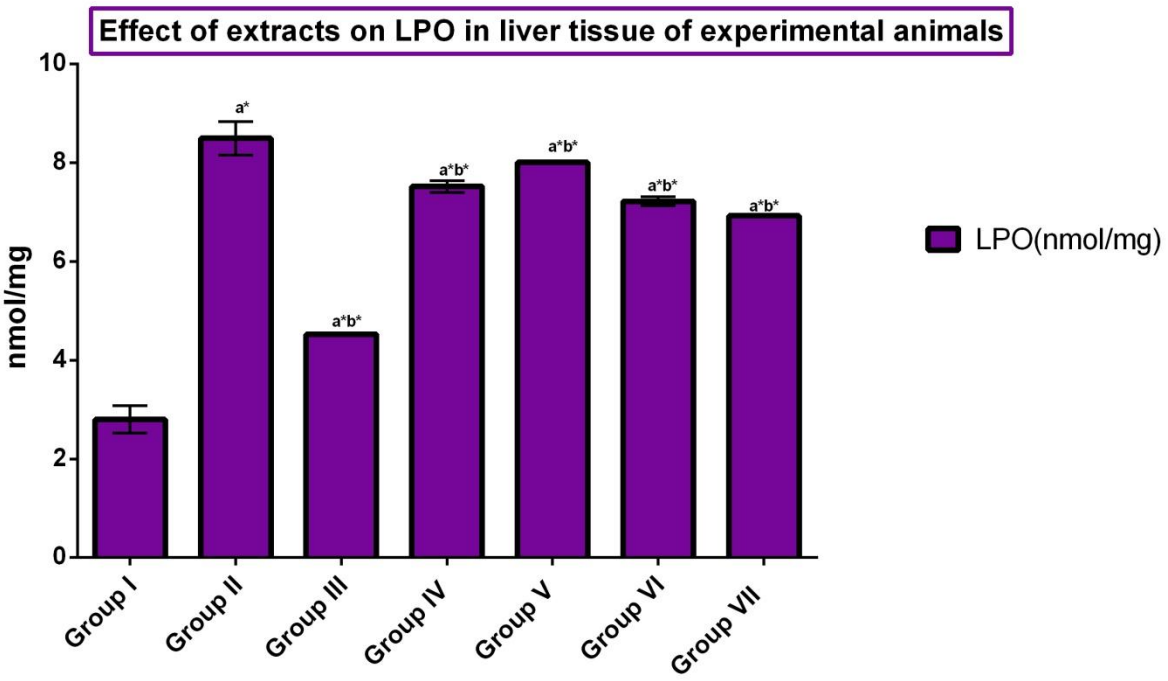

Fig2: Effect of HAEPD and HAETC on Lipid peroxidation in liver tissue of an experimental animals

A significant decrease in the level of glutathione & enzymatic antioxidants (SOD, CAT) were noted in CCl_4 induced rats. HAEPD and HAETC treated groups, the level of glutathione & activity of enzymatic antioxidants were significantly (p<0.001) reversed to normal level. The effect of HAEPD and HAETC were compared with CCl_4 intoxicated rats.

The effect of HAEPD and HAETC on rat liver lipid peroxidation, glutathione &enzymatic antioxidans (SOD, CAT) are shown in **table-2 and Figure-2**.The levels of MDA levels were considerably increased in CCl_4 treated rats compared to control rats. The pre and post treated groups with HAEPD and HAETC resulted in a significant decreased (p< 0.001) in levels of MDA and values near to normal level. Estimating the activity of serum markers enzymes like AST, ALT can make the assessment of liver function. When the liver cell is damaged, a variety of enzymes normally located in the cytosol and released in to the blood stream. The estimationof marker enzymes in serum is useful in identification of hepatocellular

damage.[33] The AST is a cytosolic enzyme, which is more specific for the liver than ALT.The transaminase has been reported to attain normal level with the healing of parenchymal and regeneration of liver cells.[34] In our study, the increased activity of AST, ALT were observed in CCl_4 induced rats compared with control rats. The elevated activities of the enzymes may be due to inflammation in the liver. Table 1 showed the administration of hydroalcohol extracts of *Pergularia daemia* and *Terminalia catappa* with significant decreased ($p < 0.001$) the values of AST and ALT. LDH is an intracellular enzyme & catalyses the readily the reversible reaction involving oxidation of lactate to pyruvate .High concentrations of LDH are found in the liver. Elevation in total serum LDH activity is used as diagnostic markers of the organ disfunction.[35]

CCl_4 treatment significantly elevated the lipid peroxidation as evident from the increased MDA level in the liver tissue. The antioxidant enzymes GPx, GST, SOD and CAT were significantly reduced in CCl_4 intoxication, besides reduction of GSH. GSH is a co-factor for several detoxifying enzymes of oxidative stresses such as glutathione peroxidase and glutathione -S- transferase are scavenges hydroxyl radicals and singlet oxygen species directly and detoxifying hydroperoxides and lipid peroxides.[36] Glutathione, synthesized from liver is the major source of plasma antioxidants. [37] It can also regenerate some of most important antioxidants such as vitamin C and E. And hence, it can be assumed that liver damage resulted in reduction in glutathione level and the antioxidant enzymes. Beddowes *et al.*,[38] showed that CCl_4 increased lipid peroxidation and depletion of glutathione. The significant reduction of hepatic gluatathione , glutathione peroxidase and glutathione –S-transferase in CCl_4 administrated rats was reported.[39]

Histopathological examination of liver of the normal animals showed the histological structure with central vein surrounded by normal hepatocytes as shown in “Fig 3”. The histopathological studies are the evidence of efficacy of plant extracts as protectant. In CCl_4 intoxicated rats showed severe fatty changes & inflammation with micro and macro vesicular status as shown in “Fig-4”. The liver of CCl_4 intoxicated with silymarin showed reduced inflammation and fatty changes as shown in “Fig-5”.While liver of CCl_4 intoxicated with pre treated HAEPD and HAETC showed fatty changes and inflammation as shown in “Fig-6,7”. Finally the liver of CCl_4 intoxicated with post treated HAEPD and HAETC showed in “Fig-8,9” with central vein and hepatocytes. Post treated animals with the plant extracts are used to reverse the inflammation & fatty changes and improve the histopathology.

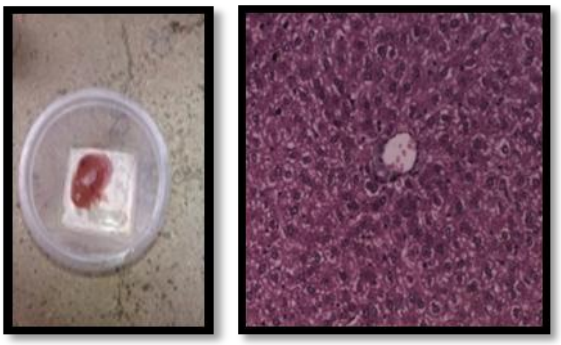

Fig-3

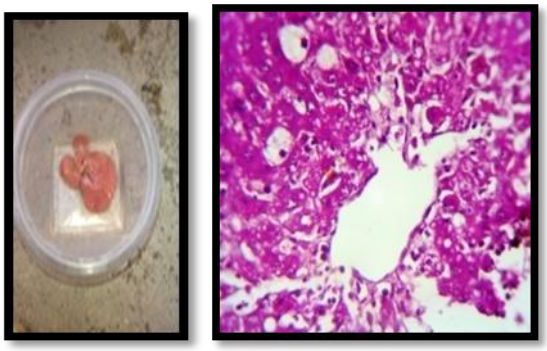

Fig-4

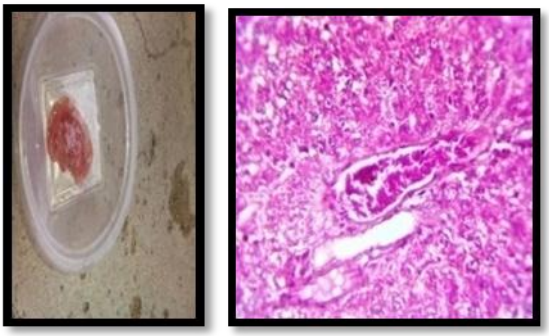

Fig-5

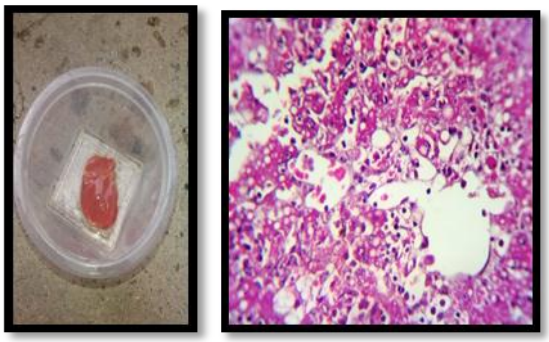

Fig-6

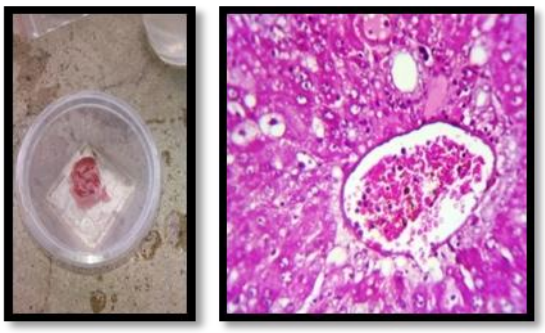

Fig-7

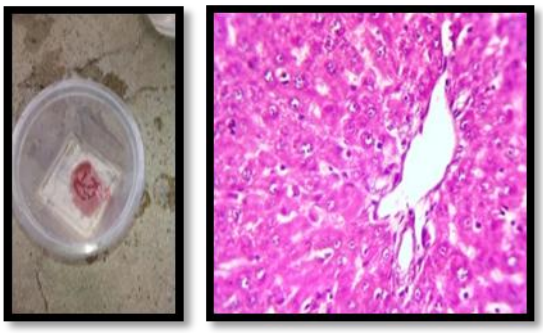

Fig-8

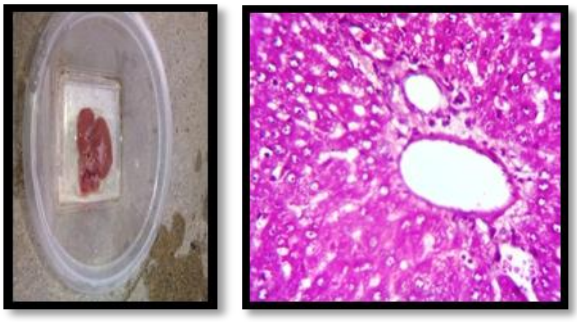

Fig-9

PHOTOS

Fig-3: Group I(Control)-Normal liver cells., Fig-4: Group II(CCl_4 Treated liver cells, Fig-5: Group III(CCl_4+Silymarin), Fig-6: Group IV(HAEPD+CCl_4), Fig-7: Group V

(HAETC+CCl_4), Fig-8: Group VI(CCl_4+ HAEPD), Fig-9: Group VII(CCl_4+ HAETC)-(X-100).

CONCLUSION

The present findings demonstrate the hepatoprotective and antioxidant activities of HAEPD and HAETC in the experimental animal model. From the biochemical and antioxidants activity and histopathological assays drastic changes and restoration was observed in post treatment with HAETC than the post treatment with HAEPD and pre treatment groups. Further work need to be done to isolate & purify the active principle involved in the hepatoprotective activity of the HAEPD & HAETC.

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