A COMPARATIVE STUDY OF *Justicia adhatoda*, *Mimosa pudica* AND *Vitex negundo* AGAINST HEPATOPROTECTIVE ACTIVITY IN ALBINO RATS - *IN VIVO* EVALUATION

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Summary

Aim of the study was to investigate the comparative effect of ethanolic extracts of *Justicia adhatoda, Mimosa pudica* and *Vitex negundo* on hepatoprotective activity against Perchloroethylene induced liver damage in albino rats. Liver damage was induced by administration of Perchloroethylene (1000 mg/kg body weight). All the tested extracts showed potent hepatoprotective activity at 1000 mg/kg body weight test dose which was comparable with that of the standard silymarin used in similar test dose. The ethanolic extract was able to restore the biochemical levels to normal which were altered due to Perchloroethylene intoxication in albino rats.

Keywords: *Justicia adhatoda, Mimosa pudica*, *Vitex negundo*, Ethanolic extract, Perchloroethylene, Hepatoprotective.

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Introduction

Perchloroethylene (tetrachloroethylene, Perc) is a solvent used in dry cleaning operations and industrial applications such as metal degreasing. It is classified as a group 2A carcinogen (probably carcinogenic to humans) by IARC [1]. Perc has been found to produce increases in hepatocellular carcinomas and/or adenomas in mice in chronic inhalation bioassays [2, 3] and is classified as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program [4] and "probably carcinogenic to humans" by the International Agency for Research on Cancer. Perc is metabolized primarily to trichloroacetic acid (TCA), which is also a mouse hepatocarcinogen [5, 6, 7, 8]. Higher incidence of nephropathy [9] and urinary tract cancer [10] were observed in dry cleaning workers exposed to Perc. Animal studies showed that Perc is hepatocarcinogenic in both genders of B6C3F1 mice [11] and a kidney carcinogen in rats [12].

The hepatotoxic, nephrotoxic and carcinogenic effects of Perc depend on its metabolism to reactive metabolites [13&14]. Cytochrome p450 dependent oxidation and glutathione (GSH) conjugation are two principal pathways of Perc metabolism that occur in liver and kidney of mice [15] leading to the generation of reactive metabolites which may covalently bind to cellular macromolecules [16]. Perc oxidation is catalyzed primarily by CYP2E1 to form Perc-epoxide and further to trichloroacetyl chloride, which can react with amino groups in macromolecules resulting in hepatotoxicity or with water to give trichloroacetic acid (TCA) [17].

Justicia adhatoda L. used against various respiratory disorders such as Anti asthmatic, Antispasmodic (respiratory tract), Its Bronchodilator, Expectorant (relaxing), Oxytocic activities have been reported. *Vitex negundo* L. is a large, aromatic shrub, has anti-inflammatory [18], antibacterial [19], antifungal [20,21] and analgesic [22,23] activities. In contemporary medicine, *Mimosa pudica* L. is being investigated for its potential to yield novel chemotherapeutic compounds. It contains an alkaloid called mimosine, which has been found to have potent antiproliferative and apoptotic effects [24].

Recently there have been many studies on traditional medicines, attempting to develop new drugs for hepatitis from them. The hepatoprotective effects of *Justicia adhatoda vasica* aqueous leaf extract on D-galactosamine-induced liver damage in rats have been investigated [25]. The hepatoprotective effect of *Vitex negundo* against carbon tetrachloride-induced liver damage have been investigated [26]. The hepatoprotective Activity on *Vitex negundo* Linn. (Verbenaceae) by using Wistar Albino Rats in Ibuprofen Induced Model have been investigated [27]. Hepatoprotective activity of *Vitex negundo* leaf extract against Anti-tubercular Drugs induced hepatotoxicity also has been investigated [28]. Hepatoprotective effects of 50% ethanolic extract of *Mimosa pudica* against CCl4 induced hepatotoxicity in rats have been investigated [29].Hepatoprotective activity of *Mimosa pudica* leaves against carbon tetrachloride induced toxicity also have been investigated [30].

In this study, to evaluate comparative studies of ethanolic extracts of *Justicia adhatoda, Vitex negundo and Mimosa pudica* against perchloroethylene induced hepatotoxicity in rats.

Materials and Methods

Plant material

The medicinal plants selected for present investigation *Justicia adhatoda, Vitex negundo and Mimosa pudica* were collected from Kerala and Tamil Nadu. The plant was taxonomically authenticated by Dr.G.V.S.Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore, Tamilnadu, India. The voucher No. BSI / SRC / 5 / 23 / 10-11 / Tech. 297, BSI / SRC / 5 / 23 / 10-11 / Tech. 296, BSI / SRC / 5 / 23 / 10-11 / Tech. 295.

Preparation of plant extract

Shade-dried powder (100g of *Justicia adhatoda, Vitex negundo and Mimosa pudica)* was taken and suspended in 500 ml of 99% ethanol. The crude extract obtained was condensed using rotary evaporator to dryness [31].

Phytochemical screening

Phytochemical screening was done for analyzing secondary metabolites that are responsible for curing ailments. The phytochemical screening of the plant extract was carried out by the following methods of [32&33].

Animals used

Female Wistar albino rats of (100-150 gm) were maintained under control conditions of light and temperature (25°C+1°C) in animal house of Karpagam University, Coimbatore. Standard animal food pellets and water *ad libitum* were provided. The study was approved by the Institutional Animal Ethics Committee (IAEC), Govt. of India.

Experimental design

The animals were divided into nine groups

Group I	-	Control animals
Group II	-	Perc induced animals (1000 mg/kg body wt)
Group III	-	Perc induced animals treated with Ethanolic extract of *Justicia adhatoda* (250mg/kg body wt)
Group IV	-	Perc induced animals treated with Ethanolic extract of *Mimosa pudica* (250mg/kg body wt)
Group V	-	Perc induced animals treated with Ethanolic extract of *Vitex negundo* (250mg/kg body wt)
Group VI	-	Ethanolic extract of *Justicia adhatoda* alone (250mg/kg Body wt)
Group VI	-	Ethanolic extract of *Mimosa pudica* alone (250mg/kg Body wt)
Group VIII	-	Ethanolic extract of *Vitex negundo* alone (250mg/kg Body wt)
Group IX	-	Positive Control (Silymarin 25 mg/ kg body wt)

After the experimental period, the animals were sacrificed under light chloroform anesthesia. Blood was drawn from the Para-orbital venous complexes, blood, serum and heart was separated. The heart was excised immediately, cleaned free of extraneous material and perfused with ice cold saline (0.9%) which are used for biochemical estimations and also stored in 10% formalin, which are used for histopathological studies respectively.

At the end of 10^{th} day, blood was withdrawn and collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the estimation of AST, ALT and ALP by the method of King [34]. The liver sample was taken and homogenized and the assay of tumor marker enzymes and antioxidant enzymes were conducted. Estimation of protein was also done.

Statistical analysis

The data expressed as the mean ± SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). Statistical significance was considered at $p<0.05$.

Results

Table 1 shows the activities of alanine transaminase, aspartate transaminase and alkaline phosphatase in serum of normal, perchloroethylene control and treated groups. The activities of alanine transaminase, aspartate transaminase and alkaline phosphatase in serum significantly increased in perchloroethylene control group compared to normal group. The levels of the above enzymes were significantly reduced on treatment with *Justicia adhatoda, Vitex negundo and Mimosa pudica.*

Table.1. Effect of ethanol extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on serum biochemical parameters in control and experimental animals.

Groups	**ALP**	**ALT**	**AST**
Normal	145. 06 ± 13. 89^{ab}	24.72±3.07^{a}	20.87±3.76^{a}
PERC control	264.09±16.98^{f}	57.80 ±5.39^{c}	44.48±1.40^{e}
J.adhatoda +PERC	182.26±19.05^{e}	32.48± 2.11^{b}	30.86±1.01^{c}
V.negundo+ PERC	169.24±3.81^{cd}	38.04 ± 1.11^{b}	35.69±0.28^{d}
M.pudica+PERC	185.98±6.27^{de}	33.26±2.66^{b}	36.31±0.50^{d}
J.adhatoda alone	142.27±2.49^{a}	28.59±4.76^{a}	21.56±0.91^{a}
V.negundo alone	158.08±14.18^{bc}	27.48 ± 2.17^{a}	23.95±1.911^{b}
M.pudica alone	165.52±9.44^{c}	28.45 ±1.05^{a}	22.55±1.83^{ab}
Silymarin(25 mg/kg) + PERC	147.25±3.8^{ab}	25.09±1.77^{a}	20.19±0.8^{a}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-f) differ significantly at $p < 0.05$ (DMRT). Units: AST, ALT, - µmoles of pyruvate liberated/l; ALP - µmoles of phenol liberated.

Table 2 shows the level of lipid profile in liver homogenate of control and experimental groups. In perchloroethylene induced group the lipid profile levels significantly increased. The levels of the lipid profile were significantly reduced on treatment with *Justicia adhatoda, Vitex negundo and Mimosa pudica.*

Table.2. Effect of ethanol extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on lipid profile in control and experimental animals

Groups	Cholesterol	TGL	HDL	LDL
Normal	74.81±8.09^{a}	75.03±4.32^{a}	35.97±4.55^{e}	37.29±6.07^{a}
PERC control	191.83±5.88^{f}	204.32±5.39^{e}	17.25±5.37^{a}	89.67±7.16^{f}
J.adhatoda+PERC	118.79±9.41^{cd}	136.27±5.39^{d}	24.54±5.13^{b}	50.66±6.84^{ab}
V.negundo+ PERC	131.81±18.66^{d}	140.79±6.46^{d}	25.45±12.01^{b}	61.41±16.02^{bc}
M.pudica+PERC	129.75±7.19^{d}	120.05±9.18^{c}	9.91±8.31^{c}	69.85±10.92^{c}
J.adhatoda alone	99.07±2.25^{b}	96.68±5.73^{b}	33.54±2.08^{de}	40.75±2.69^{a}
V.negundo alone	101.56±4.18^{b}	94.34±3.64^{b}	35.57±4.48^{e}	44.58±5.97^{a}
M.pudica alone	107.23±4.10^{bc}	86.22±5.23^{b}	36.59±4.72^{e}	47.28±6.29^{a}
Silymarin (25mg/kg)+ PERC	75.97±1.28^{a}	76.97±1.14^{a}	34.49±1.05^{e}	39.47±0.85^{a}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-f) differ significantly at $p < 0.05$ (DMRT).

Table 3 shows the level of lipid peroxidation in liver homogenate of control and experimental groups. In perchloroethylene induced group the lipid peroxidation levels significantly increased. Peroxy radicals are important agents that mediate lipid peroxidation there by damaging cell membrane.

Table.3. Effect of ethanol extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on the levels of lipid peroxide and hydro peroxide in liver of control and experimental animals

Groups	LPO
Normal	2.73±5.82^{a}
Perc control	6.64±2.27^{f}
J.adhatoda+Perc	5.39±3.23^{e}
V.negundo+ Perc	4.81±0.23^{d}
M.pudica+Perc	4.66±0.13^{c}
J.adhatoda alone	2.91±9.22^{b}
V.negundo alone	2.88±6.22^{b}
M.pudica alone	2.73±9.89^{a}
Silymarin (25 mg/kg) + Perc	2.86±0.13^{b}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-f) differ significantly at $p < 0.05$ (DMRT).
Units: LPO - nM/mg protein.

Table.4. Effect of aqueous extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on the activities of enzymatic antioxidants in liver of control and experimental animals

GROUPS	SOD	CAT	GPx	GST	GR
Normal	7.53±0.10^{d}	1.95±3.77^{e}	2.04±4.77^{d}	103.13±2.8^{d}	10.24±1.48^{e}
PERC control	3.70±0.25^{a}	1.15±7.81^{a}	0.80±2.73^{a}	46.66±2.27^{a}	3.78±0.84^{a}
J.adhatoda+PERC	4.75±1.26^{b}	1.45±1.99^{b}	1.25±1.04^{b}	86.46±3.94^{c}	7.01±0.87^{b}
V.negundo+PERC	6.19±0.15^{c}	1.67±5.99^{c}	1.21±6.69^{b}	96.69±2.86^{d}	8.07±0.12^{c}
M.pudica+PERC	6.04±0.27^{c}	1.43±3.60^{b}	1.22±7.11^{b}	76.71±2.70^{b}	8.06±3.26^{c}
J.adhatoda alone	7.06±4.11^{d}	1.65±7.53^{c}	1.85±6.78^{c}	94.83±3.07^{d}	9.25±0.25^{d}
V.negundo alone	7.14±9.23^{d}	1.86±6.51^{d}	1.89±4.87^{c}	97.92±1.85^{d}	9.44±9.84^{de}
M.pudica alone	7.05±8.39^{d}	1.81±5.16^{d}	1.93±8.28^{c}	98.12±5.53^{d}	9.67±0.62^{de}
Silymarin (25 mg/kg)+ PERC	7.13±0.0^{d}	1.94±0.05^{e}	2.03±0.02^{d}	100.28±4.9^{d}	10.14±1.1^{e}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).
Units: SOD - inhibition of 50% nitrite formation/min/mg protein; CAT - µmol of H_2O_2 consumed/min/mg protein; GPx - µmol of glutathione oxidized/min/mg protein; GR - µmole of glutathione utilized/min/ mg protein.

Table 4 shows the levels of superoxide dismutase, catalase and glutathione peroxidase, Glutathione S-transferase, Glutathione reductase in liver homogenate of control and experimental groups. In perchloroehtylene induced group the antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase, Glutathione S-transferase, Glutathione reductase levels were significantly decreased. By the administration of ethanolic extract of *Justicia adhatoda, Vitex negundo and Mimosa pudica* to perchloroethylene induced rats the antioxidant enzyme levels were restored to normal.

Table 5 shows the level of protein in control and experimental groups. In perchloroethylene induced group the protein levels significantly decreased. The levels of the protein were restored to normal on treatment with *Justicia adhatoda, Vitex negundo and Mimosa pudica.*

Table.5. Effect of aqueous extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on protein level in control and experimental animals

Groups	Protein
Normal	192.78±5.71^{e}
PERC control	118.98±11.00^{a}

J.adhatoda+PERC	150.66±4.22^{b}
V.negundo+ PERC	147.33±1.36^{b}
M.pudica+PERC	152.00±0.89^{b}
J.adhatoda alone	177.25±2.02^{d}
V.negundo alone	170.55±3.61^{c}
M.pudica alone	175.02±2.28^{cd}
Silymarin (25 mg/kg)+PERC	193.17±1.44^{e}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Table 6 shows the concentration of Non - protein nitrogenous compounds such as Urea, Creatinine found to be significantly increased and decreased the Uric acid level in perchloroethylene induced group. The levels of the Urea, Creatinine reduced and increased the level of Uric acid on treatment with *Justicia adhatoda, Vitex negundo and Mimosa pudica.*

Table.6. Effect of ethanol extracts of *Justicia adhatoda, Mimosa pudica and Vitex negundo* on serum biochemical parameters in control and experimental animals

Groups	**Urea**	**Uric acid**	**Creatinine**
Normal	31.25±2.40^{a}	8.70±0.34^{de}	3.20±0.14^{a}
PERC control	70.83±9.62^{e}	3.18±0.34^{a}	11.25±1.44^{d}
J.adhatoda +PERC	45.83±4.81^{c}	6.24±0.96^{b}	7.04±0.43^{c}
V.negundo+ PERC	36.45±1.20^{ab}	6.36±0.27^{b}	6.50±0.76^{bc}
M.pudica+PERC	40.41±0.96^{bc}	6.18±0.34^{b}	5.83±9.62^{b}
J.adhatoda alone	31.25±2.40^{a}	7.92±0.13^{cd}	3.29±0.14^{a}
V.negundo alone	34.16±1.44^{ab}	7.80±0.83^{c}	3.62±0.14^{a}
M.pudica alone	37.70±1.20^{ab}	6.93±1.86^{e}	3.58±0.38^{a}
Silymarin (25 mg/kg) + PERC	31.91±3.35^{a}	8.5±0.22^{de}	3.25±0.26^{a}

Values are expressed as mean ± SD for twelve animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Discussion

In the present study it was observed that the animals treated with perchloroethylene resulted in the significant hepatic damage as shown by the elevated levels of marker enzymes such as ALT, AST and ALP. Excessive absorption of PCE can produce depression of the central nervous system and hepatic and renal damage [35]. When liver cell plasma is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes [36].

Metabolism of perchloroethylene (Perc) occurs by cytochrome p450-dependent oxidation and glutathione (GSH) conjugation. The cytochrome p450 pathway generated tri and dichloroacetate as metabolites of Perc, and these are associated with hepatic toxicity and carcinogenicity.

Previous studies have shown that administration of dichloroacetate and trichloroacetate increased lipid peroxidation, when administered acutely to naive animals [37]. This also induces a variety of changes in intermediary metabolism, but their effects are quite distinct in the fact that dichloroacetate has major effects on carbohydrate metabolism, whereas tri-chloroacetate and other peroxisome proliferators primarily affect lipid metabolism [38, 39].

Free radical production in cells is relatively low in normal conditions, given the various and very active defense systems including enzymatic and nonenzymatic antioxidant enzymes administration of perchloroethylene caused a significant inhibition of glutathione peroxidase [40]. A defective antioxidant defense system in PER administered mice was evidenced by the low level of enzymic antioxidants (SOD, CAT, GPx, GST, and GR). SOD is an endogenous enzymatic scavenger which can counterbalance the oxidative destruction of free radicals. Most of the SOD in tissues of cytoplasmic origin and contains Cu and Zn an essential prosthetic groups. The decrease in SOD after perchloroethylene administration may be attributable to an interaction between Cu and Zn with high levels of perchloroethylene in tissues. Potential hepatoprotective agents therefore include either free radical scavenging property or agents which are capable of augmenting the activity of antioxidant enzymes (SOD, CAT). In our present study treatment with ethanolic extract of *Justicia adhatoda, Mimosa pudica and Vitex negundo* the antioxidant enzyme levels significantly increased. The leaf extract of *Adhatoda vasica, Vitex negundo* and *Mimosa pudica* is having antilipid peroxidant effect [41, 42, 43]. The present study revealed that *Justicia adhatoda, Mimosa pudica and Vitex negundo* also has potent antilipid peroxidant effect.

In the present study, it is observed that the bioactive fractions present in *Justicia adhatoda, Mimosa pudica and Vitex negundo* are responsible for the marked hepatoprotective effects, observed in present study.

In conclusion, the result of this study seems to confirm that the ethanolic extract of *Justicia adhatoda, Mimosa pudica and Vitex negundo* have potent hepatoprotective action upon perchloroethylene-induced hepatic damage in rats and possess antilipid peroxidative and free radical scavenging activities. The present study thus justifies the

traditional use of *Justicia adhatoda, Mimosa pudica and Vitex negundo* in the treatment of liver disease and also point out that *adhatoda, Mimosa pudica and Vitex negundo* future detailed investigation as a promising hepatoprotective agent.

On the basis of results obtained in the present study, it can be concluded that *Justicia adhatoda* has high hepatoprotective activity than *Mimosa pudica* and *Vitex negundo*.

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