

## Research Article



## Hepatoprotective Effect and *In Vivo* Antioxidant Activity of Stem Barks of *Hibiscus vitifolius* Linn against Paracetamol Induced Hepatotoxicity in Rats

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### ABSTRACT

The objective of this study was to evaluate the hepatoprotective activity and *in vivo* antioxidant activity of *Hibiscus vitifolius* Linn stem barks against paracetamol induced hepatotoxic model. Dried stem barks were coarsely powdered and extracted with petroleum ether, chloroform, ethyl acetate and methanol by successive extraction in a soxhlet apparatus for 72 h. Preliminary phytochemical test of ethyl acetate extracts of *Hibiscus vitifolius* (EAHV) shows the presence of carbohydrates, phenolic compounds and flavonoids. Ethyl acetate extracts was found to be safe upto a dose of 2000mg/kg. The EAHV showed a significant dose dependent (50 mg/kg & 100 mg/kg p.o) were evaluated for their possible hepatoprotective and *in vivo* antioxidant potential. All the parameters were compared with control. There was a significant ( $p < 0.01$ ) reduction in the level of AST, ALT, ALP, total and direct bilirubin, where as an increase was found in total protein. In the tissue anti-oxidant studies, we found a significance increase in the levels of catalase (CAT), superoxide dismutase (SOD) and GPx where as there was marked reduction in the level of malondialdehyde, thiobarbituric acid reactive substance. Histology of liver sections of the animals treated with the ethyl acetate extracts showed significant reduction of necrosis and fatty formation when compared with control specimen. The effect of EAHV was comparable to that of standard drug silymarin.

**Keywords:** *Hibiscus vitifolius* Linn, ethyl acetate extract, *In vivo* antioxidant activity, hepatoprotective effect.

### INTRODUCTION

The vital organ in the body, liver plays a significant role for the metabolism of endogenous and exogenous agent. Even though there is a greater chance of liver damage occurs, drug elimination and detoxification has been done by the liver<sup>1</sup>. Hepatitis is one of the most common liver associated and prevalent disease in the world, and in most hepatitis suffers it seems to be associated with jaundice. It well known that free radical cause cell damage through mechanism of covalent binding and lipid peroxidation with subsequent tissue injury<sup>2</sup> free radicals, from both endogenous and exogenous sources are implicated in the etiologic of several degenerative diseases such as coronary artery diseases, strokes, rheumatoid arthritis, diabetes and cancer<sup>3</sup>. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals<sup>4</sup>. Phenolic compounds could be considered as natural antioxidants with potential applications in the protecting liver<sup>5</sup>. There is a great deal of interest in edible plants that contain antioxidants and health promoting phytochemicals, in view of their health implications. The extract of *Hibiscus vitifolius* is traditionally used for the treatment of jaundice and associated liver damage in india<sup>6,7</sup>. However, no literature was found on the hepatoprotective action of the ethyl acetate extract of stem barks of *Hibiscus vitifolius* Linn. In the present study we aim to evaluate the *In vivo* antioxidant and hepatoprotective activity of ethyl acetate extract of stem barks of *Hibiscus vitifolius* Linn.

### MATERIALS AND METHODS

#### Plant Material

The stem bark of *Hibiscus vitifolius* (L) (Malvaceae) was collected during the month of December 2011 from Thanjavur of Tamilnadu and authenticated by Dr.G.V.S Murthy, Scientist 'F' & Head of office, Botanical survey of India, Southern Regional Centre, Coimbatore, Tamilnadu. A voucher specimen number BSI/SRC/5/23/2011-12/Tech-1057(*Hibiscus vitifolius* Linn) was kept in Department of Pharmaceutical Analysis, JKKNCP, Kumarapalayam, India for future reference.

#### Preparation of Plant Extracts

The stem bark of *Hibiscus vitifolius* (L) (1.5 kg) was washed with water, dried in shade and crushed to power and then successively extracted with petroleum ether (60-80°C), chloroform, ethyl acetate and methanol using soxhlet apparatus upto 72 hrs<sup>8</sup>. Then the solvent was subjected to distillation and concentrated the extracts. The extracts were concentrated with rotary evaporator under reduced pressure and the dried extract was weighed and the yield of the extracts was calculated. The ethyl acetate extracts of *Hibiscus vitifolius* (EAHV) (18.46g) was used for this study. Phytochemical studies EAHV was subjected to preliminary phytochemical study<sup>9</sup>.

#### Acute Toxicity Studies

The acute toxicity study for EAHV was performed using Wistar Albino rats as per the OECD guidelines (No.425). The animals were fasted for overnight prior to the



experiment and were administered with single dose of extracts dissolved in 5 % gum acacia (doses ranges from 500 – 5000 mg/kg at various dose levels) and observed for mortality up to 48 hrs (short term toxicity). The LD<sub>50</sub> was thus determined and 1/20<sup>th</sup> of LD<sub>50</sub> value was taken as ED<sub>50</sub> value, which was selected for the hepatoprotective animal study. The animals were kept under observation up to 14 days after the drug administration to find out any delayed mortality.

### Experimental Animals

The Wistar Albino rats (both sex; 150-200gm) were procured from our institution. The animals were housed in group of 6 rats per polypropylene cages and maintained under standard laboratory condition at 22 ± 2°C in a light controlled room (12 h light/dark cycle) and were provided commercial pellet diet (Amruth feed suppliers, Maharashtra), purified drinking water ad libitum. The study was approved by institutional animal ethical committee (IAEC) constituted for the purpose of CPCSEA, Govt of India (PRIST/IAEC/Ph.D pharma-08/2012-13).

### Paracetamol Induced Hepatotoxicity

#### Experimental Design

The rats were divided into five groups of 6 rats each and treated orally as below for 14 days. Group I : Normal control

Group II: Toxicant (paracetamol 1g/kg b.w p.o)

Group III: EAHV (50 mg/kg b.w p.o)

Group IV: EAHV (100 mg/kg b.w p.o)

Group V: Served as standard (silymarin 50 mg/kg b.w p.o)

The extract is given once daily oral administration up to 14 days. On day 15<sup>th</sup> paracetamol was administered at the dose of 1000mg/kg orally to all group except group I. Animals were sacrificed under light ether anesthesia 24-h after the administration of hepatotoxin paracetamol. The blood was obtained from all groups of rats by puncturing retro orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2000 rpm at 30°C for 15 min and biochemical investigations were carried out. Liver was dissected out and used for histopathological studies and antioxidant studies<sup>10</sup>.

### Assessment of Liver Function

#### Biochemical Determinations

The biochemical parameters like serum transaminases viz., serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), Total bilirubin (TBIL), Total protein (TP) albumin and globulin according to the reported methods<sup>11-13</sup>. Protein concentration was

estimated according to the standard method using bovine serum albumin (BSA) as a standard<sup>14,15</sup>.

Estimation of MDA, lipid peroxidation (LPO), SOD, CAT, GPX, GST and GSH Liver homogenates (5% w/v) were prepared in cold 50mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000 rpm for 10 using a Remi refrigerated centrifuge. The supernatant obtained was used for the estimation of malondialdehyde (MDA)<sup>16</sup>, superoxide dismutase (SOD)<sup>17</sup>, catalase (CAT)<sup>18</sup>, reduced glutathione (GSH)<sup>19</sup>, glutathione Peroxidase (GPx)<sup>20</sup>, Glutathione-S-transferase (GST)<sup>21</sup>, lipid peroxidation (LPO) it includes thiobarbituric acid reactive substance (TBARS)<sup>22</sup> and hydroperoxides (HP)<sup>23</sup>.

### Histopathological Studies

The liver tissue was dissected out and fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin, serial sections were cut, and each section was stained with hematoxylin and eosin. The slides were examined under light microscope, and photographs were taken<sup>24</sup>.

### Statistical Analysis

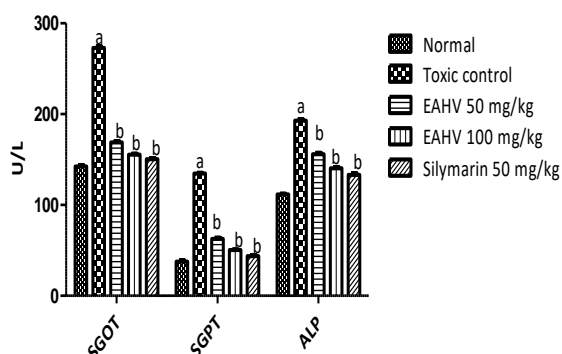
The results were expressed as mean ± SEM. Statistical analysis was carried out by using one way ANOVA followed by Dunnett's multiple tests. Statistical analysis done by the Graph pad prism 5.03 software. A value of P<0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

Continuous hot percolation of the powdered stem bark material with petroleum ether, chloroform, ethyl acetate and methanol yielded 24.92gm, 13.64gm 18.46 gm and 112.50gm of respective extracts in semisolid consistency. All the extracts were yellow to yellow brown in colour. Qualitative estimation of phytoconstituents in EAHV revealed the presence of carbohydrates, phenolic compounds and flavonoids. In acute toxicity studies, the ethyl acetate extract was found to be safe up to 2000mg/kg. No mortality or toxic symptoms were observed during the entire duration of the study. A remarkable elevation was observed in serum and tissue SGOT, SGPT, ALP and total bilirubin (272.96 ±1.576 U/L, 134.64 ±0.702 U/L, 192.99 ±1.145 U/L & 0.72 ±0.020 mg/dl) activities following paracetamol intoxication in the rat. In the groups orally treated with 100 mg/kg of the EAHV extract, the above enzyme activities were found to be decrease (155.46 ±0.799 U/L, 50.27 ±0.727 U/L, 140.47 ±1.099 U/L & 0.22 ±0.005 mg/dl) when compared to paracetamol treated group. A significant decrease (5.99 ±0.008 g/l, 3.62 ±0.072 g/l & 2.37 ±0.075 g/l) was observed against control in serum and tissue such as total protein, albumin and globulin and a significant increase (6.63 ±0.027 g/l, 4.14 ±0.023 g/l & 2.49 ±0.011 g/l) was observed on the treatment of paracetamol and EAHV respectively. The significant decrease and increase in



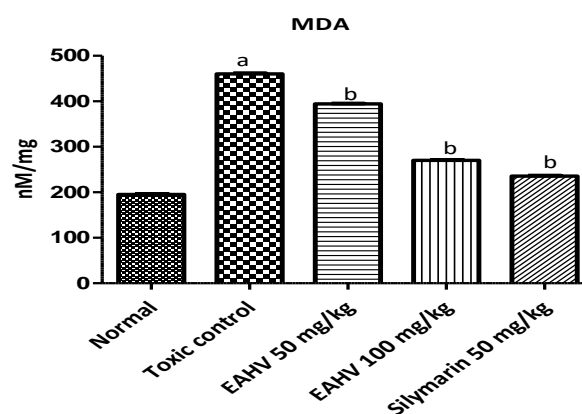
serum activities of these enzymes in this experiment could be attributed to the damaged structural integrity of the liver by paracetamol and to the hepatoprotective effectiveness of the EAHV respectively. Treatment with standard drug silymarin also reversed the hepatotoxicity significantly. The results are shown in table 1. The Effect of EAHV on SGOT, SGPT and ALP activities in experimental rats were depicted in Figure 1.



**Figure 1:** The Effect of EAHV on SGOT, SGPT and ALP activities in experimental rats.

The decrease activity of SOD, CAT, GPx, GSH and GST ( $4.21 \pm 0.158$  U/mg,  $55.26 \pm 0.565$  U/mg,  $10.13 \pm 0.206$  U/mg,  $27.95 \pm 0.793$  U/mg &  $5.14 \pm 0.141$  U/mg, the primary antioxidant enzymes are observed in the paracetamol induced rats against control which may be due to the interaction of accumulated free radicals with the associated metal ions or with the active aminoacids of these enzymes<sup>25</sup>. During hepatotoxicity these enzymes are structurally and functionally impaired by the free radicals resulting in liver damage. The EAHV and silymarin

were found to restore the levels of antioxidant enzymes, which could be due to the ability of the constituent in the administered compounds. The significant increase was observed in the levels of lipid peroxides (MDA), HP & TBARS in the supernatant of the liver homogenate of rats administered with paracetamol. The increase in the MDA, HP & TBARS of liver indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals. The EAHV retained the level of hepatic MDA, HP & TBARS to near normal control. This shows the protective action of EAHV. The results are shown in table 2. The Effect of EAHV on MDA plasma levels in experimental rats were depicted in Figure 2.



**Figure 2:** The Effect of EAHV on MDA plasma levels in experimental rats.

**Table 1:** Effects of ethyl acetate extract of *Hibiscus vitifolius* Linn on biochemical parameters in control and experimental rats.

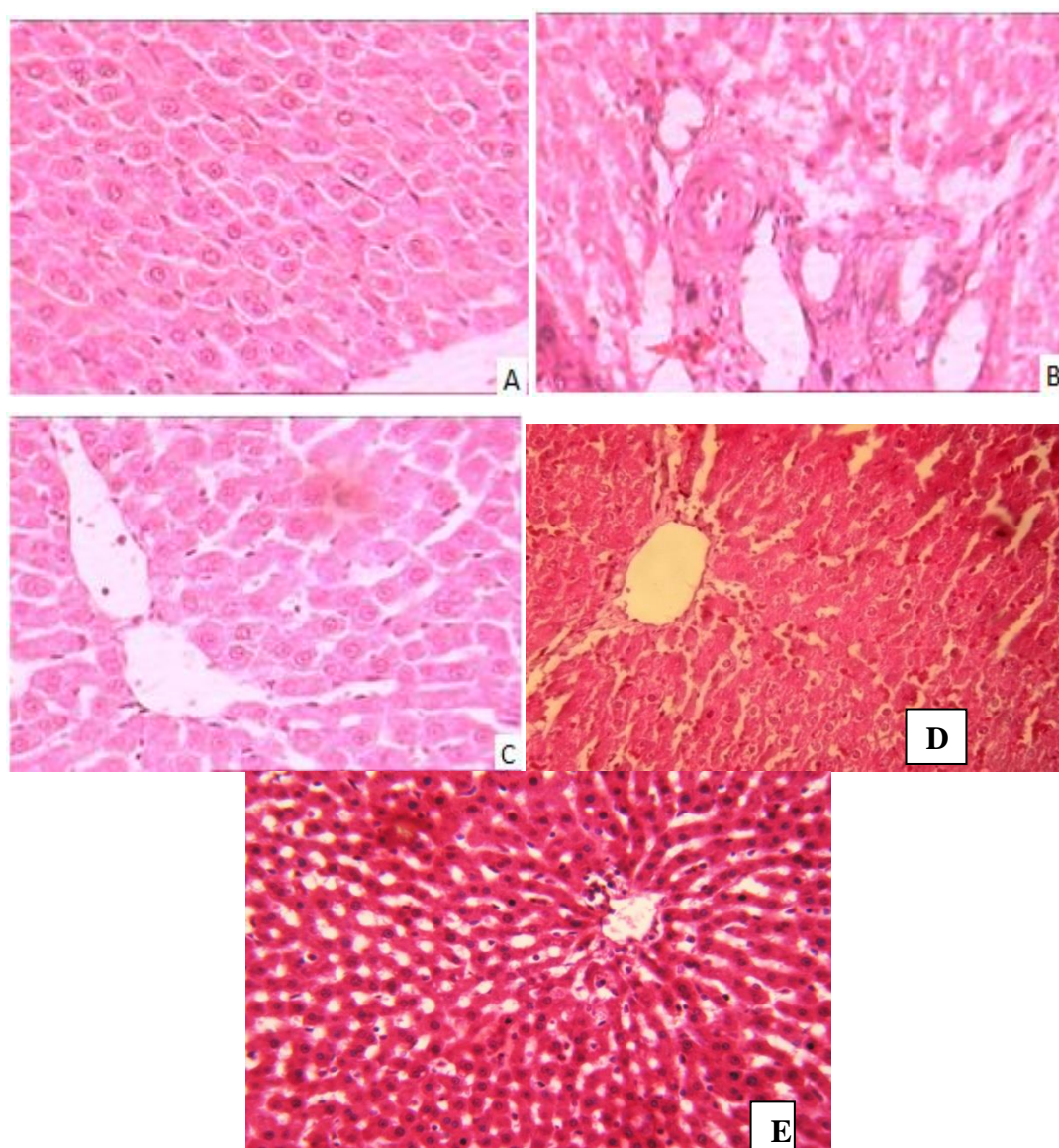
Treatment Group (G)	SGOT U/L	SGPT U/L	ALP U/L	Total Bilirubin mg/dl	Total Protein g/l	Albumin g/l	Globulin g/l
Normal(G - I)	142.32 ± 1.378	37.53 ± 1.411	111.61 ± 0.749	0.27 ± 0.008	7.09 ± 0.058	4.43 ± 0.176	2.66 ± 0.029
Toxic Control Paracetamol 1 gm/kg (G - II)	272.96 ± 1.576 <sup>a</sup>	134.64 ± 0.702 <sup>a</sup>	192.99 ± 1.145 <sup>a</sup>	0.72 ± 0.020 <sup>a</sup>	5.99 ± 0.008 <sup>a</sup>	3.62 ± 0.072 <sup>a</sup>	2.37 ± 0.075 <sup>a</sup>
EAHV 50mg/kg+paracetamol 1 gm/kg(G - III)	168.89 ± 1.330 <sup>b</sup>	62.59 ± 1.356 <sup>b</sup>	155.97 ± 1.416 <sup>b</sup>	0.38 ± 0.003 <sup>b</sup>	6.4 ± 0.024 <sup>b</sup>	3.85 ± 0.060 <sup>c</sup>	2.55 ± 0.049 <sup>b</sup>
EAHV 100 mg/kg+Paracetamol 1 gm/kg (G - IV)	155.46 ± 0.799 <sup>b</sup>	50.27 ± 0.727 <sup>b</sup>	140.47 ± 1.099 <sup>b</sup>	0.22 ± 0.005 <sup>b</sup>	6.63 ± 0.027 <sup>b</sup>	4.14 ± 0.023 <sup>b</sup>	2.49 ± 0.011 <sup>b</sup>
Silymarin 50 mg/kg+Paracetamol 1 gm/kg (G - V)	150.42 ± 1.224 <sup>b</sup>	43.7 ± 1.042 <sup>b</sup>	133.20 ± 1.754 <sup>b</sup>	0.22 ± 0.003 <sup>b</sup>	7.6 ± 0.011 <sup>b</sup>	4.25 ± 0.028 <sup>b</sup>	3.35 ± 0.008 <sup>b</sup>

One way analysis of variance (ANOVA): The data were expressed as mean ± SEM (n=6): when compared with control group - a=P <0.001. When compared with toxic control-b=P <0.001, c=P <0.05.

**Table 2:** *In vivo* anti-oxidant activity of ethyl acetate extract of *Hibiscus vitifolius* in livers of control and experimental rats.

Treatment group	MDA nM/mg	TBARS mM/100g of wet tissue	HP mM/100g of wet tissue	SOD U/mg protein	CAT U/mg protein	GPx U/mg protein	GSH U/mg protein	GST U/mg protein
Normal(G -I)	195.34 ± 1.248	0.74 ± 0.010	69.41 ± 0.315	10.41 ± 0.262	78.57 ± 0.346	16.01 ± 0.207	55.62 ± 0.356	8.19 ± 0.284
Toxic Control Paracetamol 1 gm/kg (G -II)	460.29 ± 0.973 <sup>a</sup>	1.61 ± 0.012 <sup>a</sup>	98.19 ± 0.551 <sup>a</sup>	4.21 ± 0.158 <sup>a</sup>	55.26 ± 0.565 <sup>a</sup>	10.13 ± 0.206 <sup>a</sup>	27.95 ± 0.793 <sup>a</sup>	5.14 ± 0.141 <sup>a</sup>
EAHV 50 mg/kg+paracetamol 1 gm/kg(G -III)	394.35 ± 0.898 <sup>b</sup>	1.34 ± 0.112 <sup>c</sup>	86.86 ± 0.619 <sup>b</sup>	6.35 ± 0.228 <sup>b</sup>	60.59 ± 0.404 <sup>c</sup>	19.36 ± 0.116 <sup>b</sup>	33.05 ± 0.318 <sup>b</sup>	6.24 ± 0.237 <sup>d</sup>
EAHV 100 mg/kg+Paracetamol 1 gm/kg (G -IV)	270.34 ± 0.957 <sup>b</sup>	1.09 ± 0.006 <sup>b</sup>	79.28 ± 0.876 <sup>b</sup>	7.45 ± 0.221 <sup>b</sup>	72.31 ± 1.123 <sup>c</sup>	20.31 ± 0.579 <sup>b</sup>	42.25 ± 0.433 <sup>b</sup>	6.87 ± 0.129 <sup>b</sup>
Silymarin 50 mg/kg+Paracetamol 1 gm/kg (G -V)	235.7 ± 1.217 <sup>b</sup>	1.02 ± 0.012 <sup>b</sup>	76.71 ± 0.748 <sup>b</sup>	9.29 ± 0.085 <sup>b</sup>	73.49 ± 1.116 <sup>b</sup>	18.69 ± 0.151 <sup>b</sup>	52.61 ± 0.348 <sup>b</sup>	9.79 ± 0.139 <sup>b</sup>

One way analysis of variance (ANOVA): The data were expressed as mean ± SEM (n=6): when compared with control group - a=P <0.001. When compared with toxic control-b=P <0.001, c=P <0.01 and d=P <0.05.

**Figure 3:** Effects of EAHV on cellular damage in liver of control and experimental groups of rats (400x).

A = Normal control (Shows normal hepatocytes), B = Intoxicated control (Shows focal lymphocytic infiltration and necrosis in liver), C = Silymarin (Shows hepatocytes with normal hepatocytes but rarely focal mild fatty change), D = EAHV 50 mg/kg (Shows reactive hyperplastic hepatocytes; binucleate cells), E = EAHV 100 mg/kg (Shows almost normal hepatocytes and occasional binucleate cells).

Histological profile of the normal animal showed normal hepatocytes with well preserved cytoplasm, prominent nucleus and central vein. There was no sign of inflammation, fatty change and necrosis in normal control animals (Group I) (Figure: 3 A). In animal administered with paracetamol alone, liver section showed marked congested central vein, sinusoid and multifocal area of necrosis, fatty changes and inflammatory cell with granular swelling (Group II) (Figure: 3 B). Treatment with EAHV of 100 mg/kg (Group III) (Figure: 3 E) was showed greater reduction of necrosis with disappearance of inflammation, binucleate cells with normal hepatocytes than 50 mg/kg (Group IV) (Figure: 3 D). However, silymarin showed normal hepatocytes with no sign of necrosis (Group V) (Figure: 3 C). A high degree of vacuolation, congested central vein, sinusoid and multifocal area of necrosis, fatty changes and inflammatory cell with granular swelling are significant features in the histology of a damaged liver<sup>26</sup>. In the present study, we confirmed the reversal of this features and the liver architecture was reformed with treatment of EAHV. Flavonoids play a major role in protecting the liver from injuries<sup>27</sup>. Thus the hepatoprotective and antioxidant effect of the ethyl acetate extract stem barks of *Hibiscus vitifolius* linn (EAHV) could be possibly due to the presence of flavonoids in them.

## CONCLUSION

On the basis of result obtained, it can be concluded that the ethyl acetate extract of *Hibiscus vitifolius* Linn (EAHV) 100 mg/kg has showed greater and potential hepatoprotective and antioxidant effect in rats than 50 mg/kg of EAHV. These results seem to support the traditional use of this plant in hepatotoxicity. Pharmacological studies are required to evaluate the exact mechanism of action and components present in it. Future work is continued for the hepatoprotective responsible components isolation and characterization from *Hibiscus vitifolius* Linn.

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