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Investigation of Hepatoprotective Properties of the Ethanolic Extract of *Careya arborea* Roxb. Bark in Paracetamol Induced Hepatotoxicity in Rats

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Authors' contributions

This work was carried out in collaboration with all authors. Author MJA designed, performed the study and analyses, managed the literature search and wrote the first draft of the manuscript. Authors MRI, MASK and KMND performed the study. All authors read and approved the final manuscript.

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ABSTRACT

Traditional plants have been utilized to manage hepatotoxicity according to recent trends. *Careya arborea* (CA) has been used in folk medicine to alleviate several diseases. In the present study, ethanolic extract of *Careya arborea* bark has been utilized to study its efficacy on paracetamol-induced hepatotoxicity on model rats.

SD Rats of either sex (150–200 gm) were divided into 5 groups containing 6 animals each. Acute hepatotoxicity was induced by paracetamol (600 mg/kg body weight) administered once daily for one week whereas the extract of the investigated plant was given orally throughout the whole experiment at 250 and 500 mg/kg body weight. Silymarin (100 mg/kg body weight) was given orally as a standard hepatoprotective drug. The degree of hepatoprotection was determined by the

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estimation of biochemical parameters like ALT, AST, ALP, bilirubin, total protein, albumin and globulin.
The increased serum levels of hepatic marker enzymes were found in the paracetamol treated group, indicating the severity of hepatocellular damage induced by paracetamol. Treatment with CA as well as standard hepatoprotective agent silymarin attenuated the increased levels of these hepatic enzymes. Body weight was improved insignificantly by CA, whereas liver weight was recovered significantly ($p<0.05$). ALT, AST, ALP as well as bilirubin levels were improved very highly significantly ($p<0.001$) by CA at 500 mg/kg dose. Also, the total protein and albumin levels were increased significantly at the dose of 500 mg/kg. The STD drug silymarin produced very highly significant ($p<0.001$) effect at 100mg/kg dose.
Changes in the biochemical parameters suggested that the ethanolic extract of *Careya arborea* bark has shown the promising hepatoprotective effect on paracetamol-induced liver damage in rats.

Keywords: Careya arborea; hepatoprotective activity; paracetamol-induced hepatotoxicity; liver enzymes.

1. INTRODUCTION

The liver is the main organ which regulates many important metabolic functions such as metabolism, secretion, storage, decomposition of red blood cells, plasma protein synthesis, hormone production, storage of vitamins and it has regenerative abilities as well [1,2]. Hepatic injury is directly associated with these altered metabolic functions [3]. Liver diseases like jaundice, cirrhosis and fatty liver are very prevalent public health problem in the world. Prevalence of chronic liver disease worldwide is 18.5% and cirrhosis is 4.5 to 9.5% with a mortality of 2 million per year [4]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [5].

A large number of drugs are reported to be potentially hepatotoxic like the first line anti-tubercular drugs namely, Rifampicin, Isoniazid and Pyrazinamide are potentially hepatotoxic drugs. Also, some frequently used NSAIDs like Acetaminophen, Nimesulide, Diclofenac, Ibuprofen; Anti-Hyperlipidemic Drugs like statins and anaesthetic agents cause hepatotoxicity [6, 7]. An acute or cumulative overdose of the analgesic drug paracetamol can lead to severe liver injury in humans and in experimental animals [8]. Paracetamol (Acetaminophen) is a powerful inducer and metabolized by cytochrome P450 system. The action of the P450 system on paracetamol produces a highly reactive quinoneimine that combines to the sulfhydryl groups of proteins. The toxicity occurs because of its reactive metabolite, NAPQI. NAPQI exerts its toxicity primarily via its oxidative effect on cellular proteins. The inactivation of proteins leads to the death of liver cells [9]. It has been reported that about 160 phytoconstituents from 101 plants have hepatoprotective activity [10]. Despite the tremendous strides in modern medicines, there is still a need for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells [11]. The biodiversity of the flora of Bangladesh is very broad, and several native Bangladeshi medicinal plant species have a long tradition of use with great phytotherapeutic potential [12]. So, research in medicinal plants is a vital sector for the discovery of promising drugs in Bangladesh [13].

Careya arborea Roxb. belongs to the family Lecythidaceae, commonly known as “Kumbhi” in Ayurveda. A small to medium-sized, deciduous tree, with spreading branches. Leaves up to 30 cm long [14,15]. Chief constituents found in *C. arborea* are lupeol, beta-sitosterol, betulinic acid, 1-[5-(1,3 benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] and piperidine [16,17]. It is reported to be useful in tumours, cough, bronchitis, toothache, wounds, dyspepsia, colic, haemorrhoids, intestinal worms, dysentery, leucoderma, epilepsy, abscesses, ulcers and eruptive fever particularly smallpox and antipruritic [18-20]. Piperine alkaloid isolated from the bark of *C. arborea* was found to possess analgesic activities. Alcoholic extract of CA showed the high presence of phenolic and flavonoid content and demonstrated marked antioxidant activity [21,22]. The present study was performed to evaluate the hepatoprotective effect of the ethanolic extract of *Careya arborea* against acetaminophen-induced liver injury in rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The plant *Careya arborea* (CA) bark was collected from Savar area and was identified and authenticated by the Dept. of Botany, Jahangirnagar University, Savar, Dhaka. The collected materials were thoroughly washed in water, cut into smaller parts and shed dried at 35° – 40°C for a week and pulverized in an electric grinder to get extractable powder. Then powder was extracted in soxhlet apparatus with ethanol (96%). Then the solvent washing the constituents of powder was collected in a container, dried with a rotary evaporator under reduced pressure to get viscous substance. Finally, a solid mass was obtained and preserved in a Petri dish in the refrigerator.

2.2 Experimental Animals

For the experiment, Sprague Dawley rats of either sex, weighing between 150-200g, were collected from the animal research lab in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for the animal experiment were approved by the institutional animal ethical committee.

2.3 Toxicity Studies

Toxicity studies of the extracts were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The extract was found to be safe till 5000 mg/kg p.o. Therefore, doses were selected as 250 mg/kg and 500 mg/kg b.w. [23].

2.4 Experimental Design for the Assessment of Liver Functions

The animal study was performed at Pharmacology Laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. The rats were housed in polypropylene cages at room temperature (27±2ºC). The rats were divided into five groups of 6 animals (n = 6) each [24].

Group I: Received water (10 mL/kg p.o.) once daily for 7 days, and served as normal control
Group II: Received water (10 mL/kg p.o.) once daily for 7 days and served as paracetamol control.
Group III: Received standard drug silymarin (100mg/ kg p.o.) once daily for 7 days, serving as STD.
Group IV and V: Received *Careya arborea* (CA) bark extract (250 and 500 mg/kg respectively) once daily for 7 days. In all groups except group-I paracetamol, 600mg/kg bw p.o was administered once daily with respective treatment according to Tabassum N and Agrawal SS (2004) with slight modification by error and trial [25].

Rats were anaesthetized using ketamine (500 mg/kg, i.p.). After sacrifice, blood samples from each group of rats were collected and the serum was separated by centrifugation. Serum samples were subjected to liver function tests of enzymes such as glutamate pyruvate transaminase (GPT/ALT), glutamate-oxaloacetate transaminase (GOT/AST) [26], alkaline phosphatase (ALP) [27], total bilirubin [28] and total protein by standard enzymatic colourimetric method.

2.5 Statistical Analysis

Statistical analysis for animal experiments was carried out using One way ANOVA following Bonferroni's post hoc test using SPSS 16.0 for windows. Data were presented as Mean ± SEM. The results obtained were compared with the vehicle-treated paracetamol control group. *p* values <0.05, <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

3. RESULTS AND DISCUSSION

Paracetamol is considered to be the safest non-steroidal antipyretic drug. It is commonly available over the counter if it is used in recommended doses; it is also capable of producing hepatic damage on consuming single overdoses or chronic low dose [29-31].

Acetaminophen (AAP) can cause hepatic necrosis in high doses by the formation of more NAPQI which binds covalently to cellular macromolecules and causes the hepatic GSH depletion [32,33,34,35]. Macromolecules like

liver enzymes that leak from the damaged tissues are released into the bloodstream and a study of these enzyme activities in plasma has been found to be of great importance in the assessment of liver damage [36,37].

The increased levels of AST and ALT are indicative of cellular damage and loss of functional integrity of the cell membrane in the liver [38]. The increase in ALP in liver disease is the result of increased synthesis of the enzyme by cells lining the canaliculi, usually either intra- or extrahepatic, which reflects the pathological alteration in biliary flow [39].

Through this study, the consumption of CA for 7 days was found to increase the body weight insignificantly whereas the liver weight was improved significantly ($p<0.05$) at the dose of 500 mg/kg in rats (Table 1).

The present study demonstrated the ability of the CA extract to decrease the ALT and AST level significantly ($p<0.001$) at 500 mg/kg dose and also the ALP level at both the doses ($p<0.001$) (Table 2). Reduction of the enhanced level of serum ALT, AST, ALP and total bilirubin by CA extract seemed to offer protection and maintain the functional integrity of hepatic cells. An abnormal increase in the levels of bilirubin in plasma indicates hepatobiliary disease and severe disturbance of hepatocellular function [40]. Prior oral administration of *Careya arborea* (CA) extract exhibited significant protection against AAP-induced

Table 1. Effect of *Careya arborea* bark on the body weight and liver weight in paracetamol-induced hepatotoxicity in rats

Group	Body Weight (gm) (Mean±SEM)		Liver Weight (gm) (Mean±SEM)
	Initial day	Final day	
Normal Control	191.33±1.20	205.17±2.44	5.57±0.16
Paracetamol Control	190.17±2.60	178.67±2.20†††	7.32±0.34†††
STD (Sylimarin 100 mg/ kg)	192.33±2.42	188.17±2.85	5.97±0.21**
CA 250 mg/kg	191.33±2.51	181.83±2.65	6.62±0.19
CA 500 mg/kg	190.50±1.95	185.00±1.51	6.15±0.29*

*N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant as compared to paracetamol control and † (p<0.05), †† (p<0.01) & ††† (p<0.001) are as compared to normal control group.*

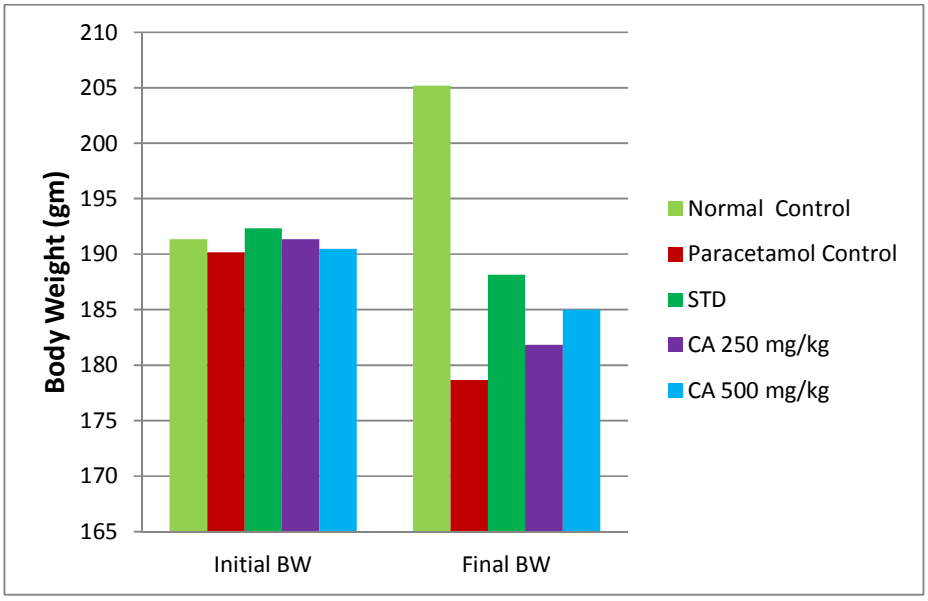

Fig. 1. Effect of *Careya arborea* bark on the body weight in paracetamol-induced hepatotoxicity in rats

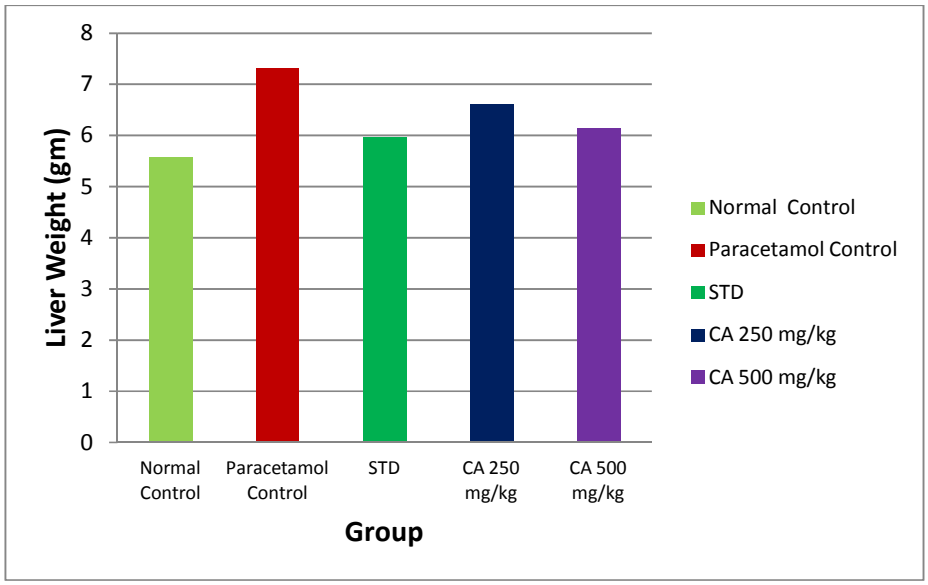

Fig. 2. Effect of *Careya arborea* bark on the liver weight in paracetamol-induced hepatotoxicity in rats

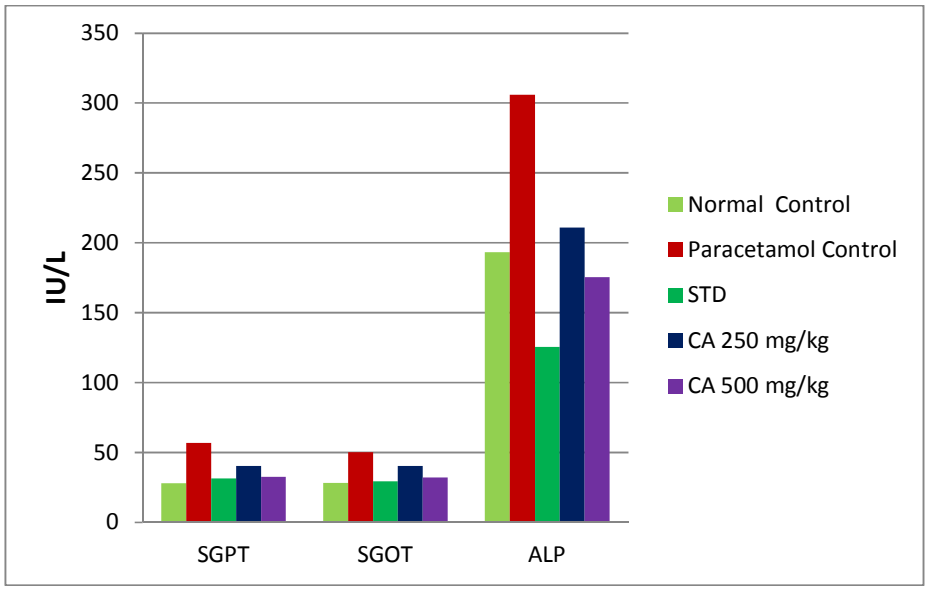

Fig. 3. Effect of *Careya arborea* bark on SGPT, SGOT and ALP level in paracetamol-induced hepatotoxicity in rats

hepatotoxicity. It decreased the levels of bilirubin significantly at both 250 mg/kg ($p<0.01$) and 500 mg/kg ($p<0.001$) which is an indication of protection against hepatic damage caused by AAP.

Most proteins found in plasma are produced by the liver, except immunoglobulins. Severe liver damage has been associated with decreased production of various proteins resulting in reduced serum levels of total protein, albumin, and/ or globulin [41,42]. Decreased protein production may render other abnormal test values. e.g. depletion of coagulation factors (all are globulins) may result in prolonged prothrombin or activated partial thromboplastin times [43]. And, increased loss of protein via urine or faeces due to renal or gastrointestinal disease will reduce serum protein levels. Inflammation anywhere in the body often results

in increased production of specific globulin proteins produced by the liver [41].

The results indicate that total protein and albumin levels were increased significantly ($p<0.01$) at 500 mg/kg dose. But plasma albumin level was decreased at both the doses although it was not significant (Table 3). The qualitative chemical tests revealed the terpenoids, flavonoids, alkaloids, saponins and tannins in the bark of *C. arborea.* The flavonoids and alkaloids may be responsible for this hepatoprotective property of this plant [44].

Table 2. Effect of *Careya arborea* bark on serum ALT, AST, ALP and bilirubin level in paracetamol-induced hepatotoxicity in rats

Group	ALT (IU/L) (Mean±SEM)	AST (IU/L) (Mean±SEM)	ALP (IU/L) (Mean±SEM)	Bilirubin (g/dl) (Mean±SEM)
Normal Control	28.00±1.42	28.17±2.97	193.33±4.26	1.03±0.11
Paracetamol Control	56.83±3.36†††	50.17±1.99†††	305.83±5.62†††	2.88±0.17†††
STD (Sylimarin 100mg/ kg)	31.33±1.87***	29.33±1.89***	125.50±6.05***	0.82±0.04***
CA 250mg/kg	40.33±3.40**	40.33±2.08	210.83±5.51***	2.03±0.14**
CA500 mg/kg	32.50±2.19***	32.00±3.09***	175.50±4.20***	1.45±0.20***

*N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant as compared to paracetamol control and † (p<0.05), †† (p<0.01) & ††† (p<0.001) are as compared to normal control group.*

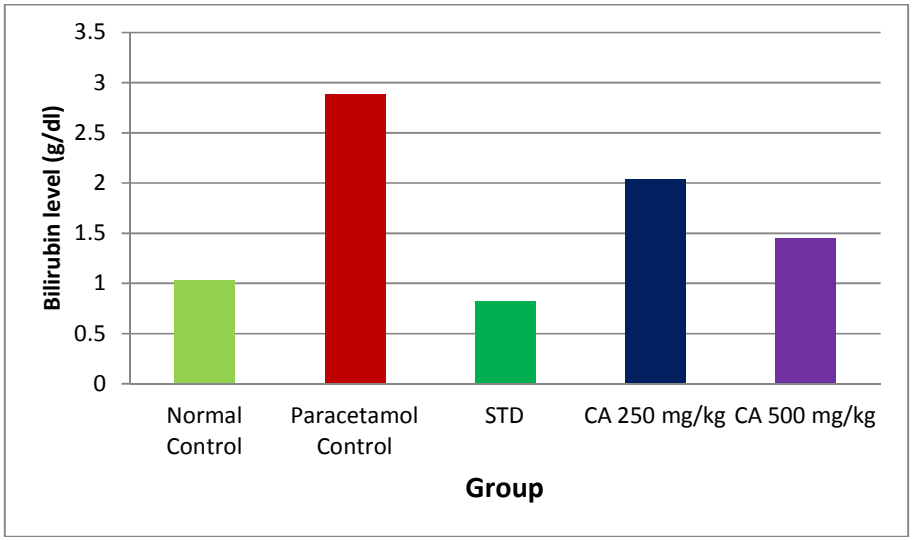

Fig. 4. Effect of *Careya arborea* bark on serum bilirubin level in paracetamol-induced hepatotoxicity in rats

Table 3. Effect of *Careya arborea* bark on serum total protein, albumin and globulin level in paracetamol-induced hepatotoxicity in rats

Group	T. Protein (g/dl) (Mean±SEM)	Albumin (g/dl) (Mean±SEM)	Globulin (g/dl) (Mean±SEM)
Normal Control	7.43±0.23	3.85±0.23	3.58±0.35
Paracetamol Control	5.23±0.25†††	2.42±0.20†††	2.82±0.42
STD (Sylimarin 100 mg/ kg)	6.38±0.20**	3.70±0.17**	2.68±0.23
CA 250 mg/kg	5.77±0.17	3.30±0.20*	2.47±0.32
CA 500 mg/kg	6.35±0.19**	3.63±0.19**	2.72±0.35

*N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant as compared to paracetamol control and † (p<0.05), †† (p<0.01) & ††† (p<0.001) are as compared to normal control group.*

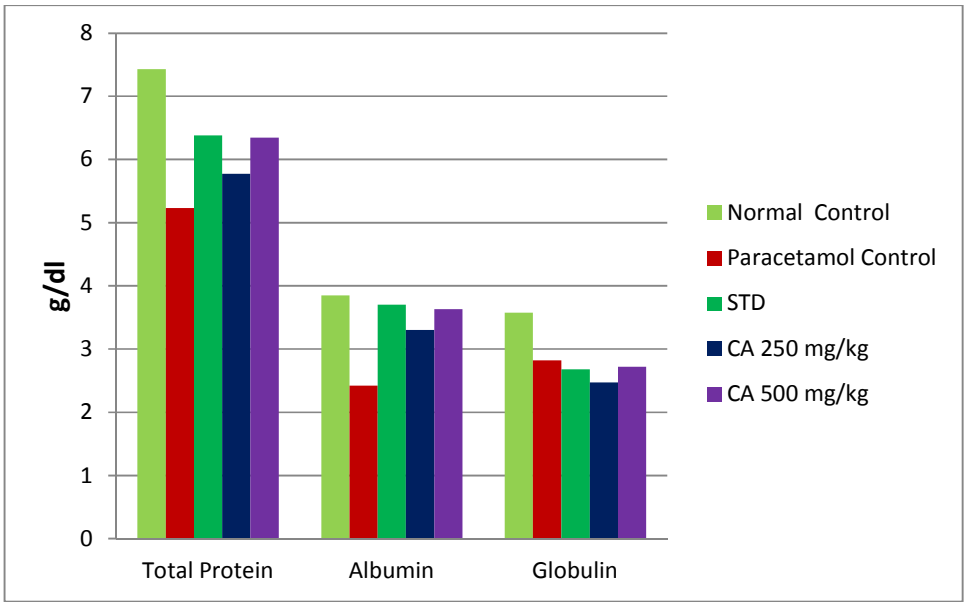

Fig. 5. Effect of *Careya arborea* bark on serum total protein, albumin and globulin level in paracetamol-induced hepatotoxicity in rats

Ambardar N and Aeri V (2013) and Kumar RS, Sivakumar T, Gupta M and Mazumder UK, (2005) also reported about the hepatoprotective effect of *Careya arborea* which ha also supportive to our study [45,46].

4. CONCLUSION

On the basis of this study, it can be concluded that the treatment with *Careya arborea* bark extract was able to protect the changes induced by AAP. Therefore, *Careya arborea* has significant value as hepatoprotective plant and was proved to be one of the herbal remedies for a liver ailment. The possible mechanism of hepatoprotective effect might be due to various phytoconstituents present in the ethanolic extract. Further studies are required to determine the more selective mechanism(s) involved and, to isolate and identify the responsible bioactive compounds for the effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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