Annals of Phytomedicine 5(1): 80-84, 2016

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN: 2393-9885

Hepatoprotective effect of *Artocarpus altilis* (Parkinson) Fosb. leaf and bark extracts against CCl_4 induced hepatic damage in albino rats

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Received May 11, 2016: Revised May 27, 2016: Accepted May 31, 2016: Published online June 30, 2016

Abstract

Medicinal plants and their formulations are being used from thousands of years in traditional medicine worldwide. Numerous plants and polyherbal formulations are used for treatment of hepatic diseases. In the present study, the hepatoprotective effect of aqueous and 80% methanol extracts of *Artocarpus altilis* (Parkinson) Fosb. leaf (ALAE, ALM80) and bark (ABAE, ABM80) were studied in rats with hepatotoxicity induced by carbon tetra chloride (CCl_4) and compared with standard drug (Liv52) and untreated groups (CCL_4). Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and selected biochemical parameters were estimated in serum. Glutathione (GSH) content and lipid peroxidation (TBARS) were analysed in serum, liver and kidney homogenates. The AST, ALT and ALP levels in extract treated groups were significantly ($p \leq 0.05$) lower than CCL_4 group. Pretreatment with ALAE, ALM80, ABAE and ABM80 restored total protein and albumin to near normal levels. The extracts improved the antioxidant status considerably as reflected by low TBARS and high GSH values. The results indicate that *A. altilis* leaf and bark extracts possess potent hepatoprotective effect against CCl_4-induced hepatic damage in rats.

Keywords: *Artocarpus altilis* (Parkinson) Fosb. 80% methanol extract, CCl_4 induced hepatotoxicity, hepatic enzymes, histopathology

1. Introduction

India has an ancient heritage of traditional medicine systems which includes Ayurveda, Siddha, Unani and Homoeopathy. The evaluation of all these drugs is based on phytochemical and pharmacological approaches which lead to drug discovery, often referred to as "natural product screening"(Chawla *et al.*, 2012). Medicinal plants, since time immemorial have been used for the treatment of various diseases all over the world. Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al.*, 2003). About 600 commercial herbal formulations claiming to exhibit hepatoprotective activity are being sold all over the world, and around 170 phytoconstituents isolated from 110 plants from 55 families, have been reported to possess hepatoprotective activity (Sharma *et al.*, 1991). However, the plants used in traditional medicine have to be pharmacologically evaluated for their safety and efficacy.

Artocarpus altilis (Parkinson) Fosb. belongs to Moraceae family, a tree of moderate size, is widely cultivated in tropics as staple crop, construction material and animal feed. The leaves have been used traditionally for the treatment of liver cirrhosis, hypertension and diabetes (Amarasinghe *et al.*, 2008), anti-inflammatory, antipyretic, infections, skin ailments, urinary tract infection and so on. Studies report the presence of flavonoids, triterpenoids and prenylflavonoids in *A. altilis* fruit (Lu *et al.*, 2007). Phytochemicals isolated from leaf tissue of bread-fruit trees are reported to be beneficial in the prevention of stroke and cardiovascular diseases (Jones *et al.*, 2011). The antihyperglycemic effect of leaf and bark extracts has been established in our laboratory (Sairam and Urooj, 2012; 2013) and studies are underway in exploring their various biological effects. With this background, in the present study, the hepatoprotective efficacy of *A.altilis* leaf and bark extracts was evaluated in CCl_4 induced Wistar strain rats.

2. Materials and Methods

2.1 Chemical and reagents

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein, albumin, urea, creatinine, total bilirubin, triglycerides, total cholesterol assay kits were purchased from Aggappe Diagnostics, Ernakulam, India. Reduced glutathione (GSH), 5,5-dithio (bis) nitro benzoic acid (DTNB) were purchased from Sigma-Aldrich, Bangalore, India. All the chemicals and reagents used in the study were of analytical grade.

2.2 Collection and preparation of samples

The leaf and bark parts of *A. altilis* were collected from Mysore district of Karnataka, India and subsequently identified by Dr. G. R. Shivamurthy, Department of Studies in Botany, University of

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Mysore, Mysore, India. The samples were washed, dried overnight (50^0C), powdered, passed through 60 mesh sieve (BS) and stored in airtight container at 4^0C untill further use.

Aqueous and 80% methanol extracts of *A. atilis* leaf and bark were used for studying their potential hepatoprotective effect against CCl_4 intoxication. The cold aqueous extracts of *A.altilis* leaf and bark were prepared by extracting powdered material with cold water (RT) in a mechanical shaker (24 h), filtered and freeze dried in freeze drier (Thermo Modulyo D, Hong Kong). Sample (15 g), was extracted with 50 ml of 80% methanol (methanol : water - 8:2 ratio, v/v) in a mechanical shaker (50 - 60 rpm) for 6 h. The extracts were evaporated at 40ºC under reduced pressure to dryness in a rotary evaporator (Superfit, India) and stored in air tight container at 4ºC until further use.

2.3 Phytochemical screening by sequential extraction

The powdered samples (20 g) were subjected to sequential extraction in a Soxhlet apparatus with various solvents, *viz,* petroleum ether (PE), benzene (BE), chloroform (CH), methanol (ME) and water (AQ) and screened for the presence of various phytochemicals such as alkaloids, flavonoids, tannins, saponins (Mojab *et al.,* 2011) triterpenoids and steroids (Trease and Evans, 1996).

2.4 Experimental animals

Adult wistar strain albino rats weighing around 140-180 g were acclimatized for 14 d. under standard conditions. The rats were housed in the polyacrylic cages, maintained at 25±2º C, 45 to 60 % RH and 12 h photo period. During acclimatization, the animals were observed for general conditions everyday. Standard pellet diet (Amrut feeds, Pune, India) and water *ad libitum* were provided. The experimental protocol of hepatoprotective studies was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) for the purpose of control and supervision of experiments on animals (UOM/IAEC/29/2011).

2.5 Methodology

2.5.1 CCl_4 *induced hepatotoxicity*

Adult Wistar strain albino rats weighing around 140-160 g were divided into various groups (Table 1), consisting of 6 animals each. The sample extracts in the form of suspensions, a standard drug Liv 52 (polyherbal tonic used antihepatotoxin) and olive oil (control and positive control) were administered orally for 7 days in the respective groups. A mixture of CCl_4 (0.5 ml/kg BW i.p) in olive oil was injected two times, at 12 and 36 h after the final administration of sample extract, and the animals were euthanized and decapitated after 12 h of final administration of CCl_4.

Table 1: Animal groups, treatment and dosage (n = 6 in each group)

Groups	Treatment	Dosage (KG^{-1} BW, p.o)
I – CON	Healthy rats-Olive oil	0.5 mL
II – PCN	CCl_4-Olive oil	0.5 mL
III - Liv52	Liv52	2.5 mL
IV – ALAE	Leaf Aqueous extract	500 mg
V - ALM80	Leaf 80% MeOH extract	500 mg
VI – ABAE	Bark Aqueous extract	500 mg
VII - ABM80	Bark 80% MeOH extract	500 mg

CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext

2.5.2 *Biochemical estimations and histopathology*

Blood was collected and serum was separated after centrifugation at 2500 ×g, for 20 min. Activities of AST, ALT and ALP were determined in serum along with estimation of total protein, albumin, urea, creatinine, total bilirubin, total cholesterol, triglycerides (TGL) using respective standard kits. The contents of Glutathione (GSH), TBARS as markers of lipid peroxidation were determined by the methods of Ellman (Ellman, 1959) and Ohkawa (Ohkawa *et al.,* 1979), respectively in serum, liver and kidney homogenates.

Various organs like liver, kidney, heart, brain and spleen were excised immediately, washed with phosphate buffered saline and weighed. Small portions of liver and kidney were fixed in 10% formaldehyde, then dehydrated in graduate ethanol (50-100%), cleared in xylene and embedded in paraffin. The sections (4-5µm) were stained with haemotoxylin and eosin (H-E) dye and examined using a photomicroscope (40x) for any histopathological changes.

2.6 Statistical analysis

The values are expressed as Mean ±SD. The data were subjected to one-way-ANOVA followed by Tukey's multiple comparisons test for significant difference ($p \leq 0.05$), using SPSS 11.5 software.

3. Results

3.1 Phytochemical screening by sequential extraction

The phytochemicals present in the extracts of *A.altilis* leaf (AL) and bark (AB) parts, were sequentially extracted in different solvents. Preliminary investigation revealed that maximum phytochemical constituents were present in methanol extracts of both leaves and bark (AL and AB). Terpenoids and triperpenoids were present in all solvent extracts of AL and AB. Saponins and tannins were present in aqueous extracts, while steroids were present in PE, BE and ME extracts of AL and AB.

Table 2: Relative body and organ weights of control and experimental groups (g) (Values in parenthesis indicate Organ-BW ratio) (n = 6)

Group	Body wt	Liver	Kidney	Heart	Brain
Con	185	5.952(3.21)^b	1.399(0.756)^a	0.683(0.369)^b	1.438(0.777)^b
CCl4	184	6.29(3.418)^b	1. 261(0.685)^a	0.585(0.317)^a	1.436(0.780)^b
LIV52	165	6.40(3.878)^b	1.40(0.848)^b	0.669(0.405)^b	1.559(0.944)^{cd}
ALAE	191	7.212(3.775)^b	1.538(0.805)^b	0.702(0.367)^b	1.484(0.776)^b
ABAE	164	6.336(3.863)^b	1.442(0.879)^b	0.653(0.398)^b	1.445(0.881)^c
ALM80	187	6.587(3.522)^b	1.395(0.745)^a	0.724(0.387)^b	1.172(0.626)^a
ABM80	212	5.677(2.677)^a	1.579(0.744)^a	0.549(0.258)^a	1.596(0.752)^b

CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80- bark 80% MeOH ext Mean values carrying different superscripts a, b, c… in columns differ significantly ($p \leq 0.05$).

3.2 Effect of extracts on CCl_4 induced hepatotoxicity

3.2.1 *Effect on hepatic enzymes and selected biochemical parameters*

The relative body weights and organ ratios are given in Table 2. The data on serum total protein, albumin, creatinine, total bilirubin, urea, total cholesterol and triglycerides is shown in Table 3. There

was no significant ($p \leq 0.05$) difference observed in biochemical parameters between the groups. The total cholesterol and triglycerides levels in positive control group were significantly high in PCN group ($p \leq 0.05$). It was interesting to note that the total cholesterol and triglyceride levels in all the extract treated groups were significantly ($p \leq 0.05$) lower than PCN group and similar to LIV52 group.

Table 3: Changes in biochemical parameters in serum of control and experimental groups (Mean ± SE)

Group	T Pro (g dL^{-1})	Albumin (g dL^{-1})	Creatinine (mg dL^{-1})	T Bilirubin (mg dL^{-1})	Urea (mg dL^{-1})	TC (mg dL^{-1})	TGL (mg dL^{-1})
Con	5.45 ±0.7	3.43±0.26	1.20±0.08	0.20±0.01	36.00±2.02	67^{a}±8.21	245^{c}±21.4
CCl4	4.55±0.5	2.52±0.18	1.80±0.31	2.10±0.06	37.00±6.98	122^{c}±5.3	206^{b}±10.8
LIV52	5.23±0.18	2.92±0.11	1.28±0.16	0.70±0.02	41.00±11.23	75^{a}±8.24	137^{a}±4.6
ALAE	4.71±0.76	3.13±0.27	1.40±0.25	1.82±0.09	45.81±13.27	62^{a}±4.8	119^{a}±7.03
ABAE	4.74±0.4	3.05±0.45	1.60±0.17	1.15±0.05	46.60±2.1	85±2.9	145^{a}±8.6
ALM80	4.45±0.38	3.00±0.21	1.68±0.22	1.80±0.1	48.10±7.03	97^{b}±9.36	123^{a}±4.75
ABM80	4.41±0.15	3.06±0.21	1.60±0.17	1.38±0.12	47.00±11.87	102^{b}±2.9	195^{b}±11.77

CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext T Pro-Total protein, T Bilirubin-total bilirubin, TC-total cholesterol, TGL-tryglicerides Mean values carrying different superscripts a, b, c… in columns differ significantly ($p \leq 0.05$).

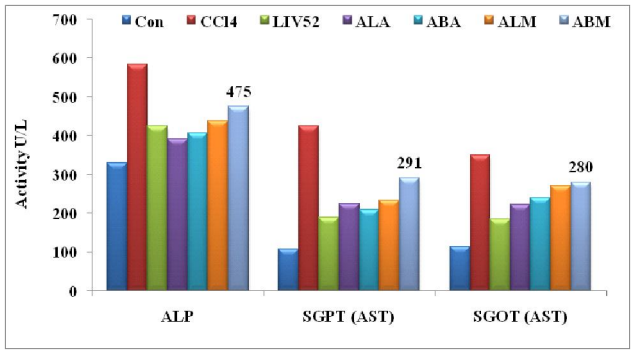
Table 4: Histopathological changes in liver of control and extract treated groups

Group	Liver architecture	Necrosis	Steatosis / Fatty change	Chronic inflammatory infiltration	Any other
CON	Normal	-	-	-	-
PCN	Destroyed	Present	Moderate - severe	Maximal	Destruction of bile duct and blood vessels
LIV52	Normal	-	Absent	Minimal	-
ALAE	Normal	Minimal	Mild	Occasional foci	-
ABAE	Normal	Minimal	Minimal	Occasional foci	-
ALM80	Normal	Minimal	Very minimal	Occasional foci	-
ABM80	Normal	Minimal	Mild - moderate	Occasional foci	-

CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext

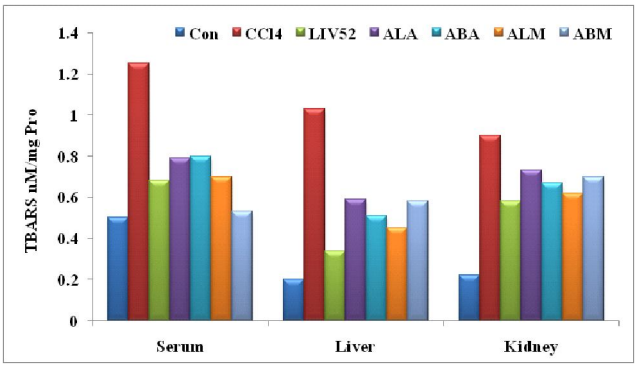
The protective effects of sample extracts against CCl_4 induced hepatic injury was assessed by analysing activity of hepatic enzymes (Figure 1). A significant ($p \leq 0.05$) difference in serum biochemical markers were observed between normal and experimental groups. Pretreatment with sample extracts significantly reduced the ALP, AST and ALT activities when compared to PCN. There was a significant decrease in ALP activity in ALAE and ABAE groups than Liv52. The serum hepatic enzymes activity was higher in ALM80 and ABM80 groups, however it was significantly lower than PCN group ($p \leq 0.05$).

The TBARS (Figure 2) and glutathione (Figure 3) levels in serum and liver and kidney homogenates were analysed in all the groups. The treatment with leaf and bark extracts did not show any adverse effects on cellular defense mechanisms against oxidative stress. The results suggested that leaf extracts performed better than the bark extracts.



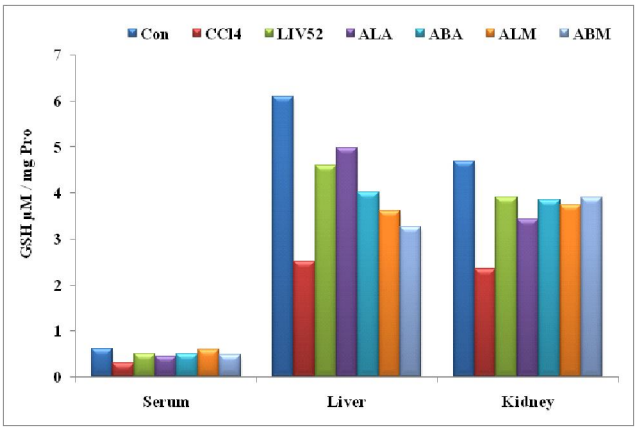
CON-Control, PCN-Positive control, LIV52-Livosin 52, ALAE- leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext Mean values carrying different super-scripts a, b, c… bars differ significantly ($p \leq 0.05$).

Figure 1: Activity of hepatic enzymes in serum of different groups (U L^{-1})



CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext Mean values carrying different superscripts a, b, c… on bars differ significantly ($p \leq 0.05$).

Figure 2: TBARS levels of control and extract treated groups (ng mg^{-1} Pro)

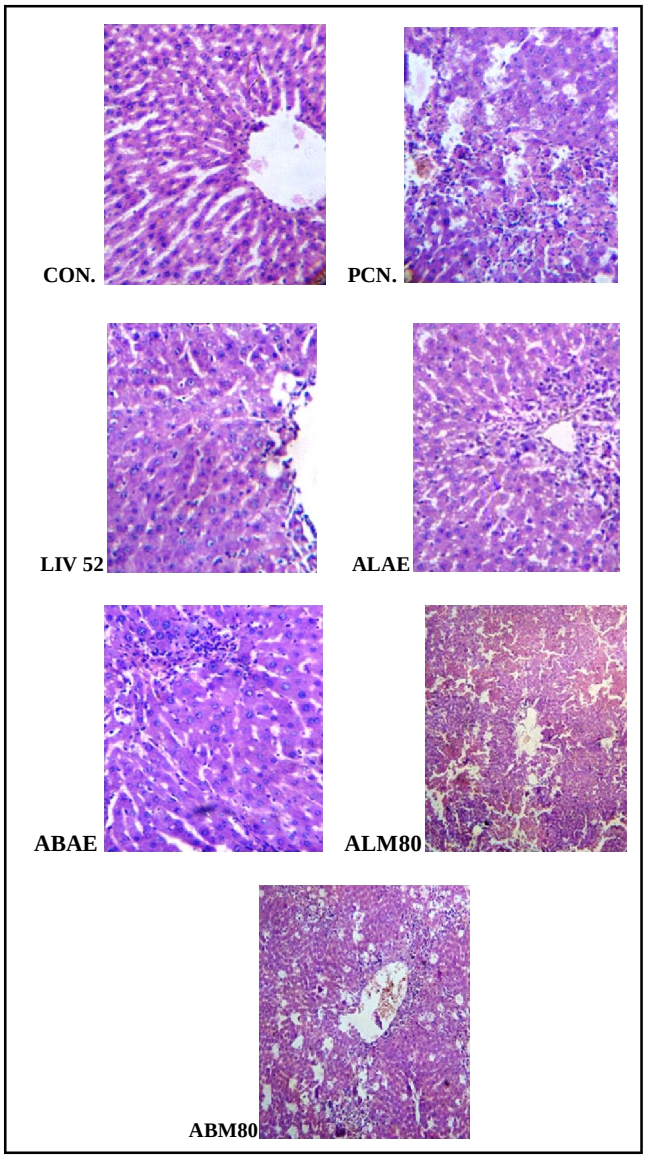


CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext Mean values carrying different super scripts a, b, c… on bars differ significantly ($p \leq 0.05$).

Figure 3: Glutathione levels of control and extract treated groups (µM mg^{-1} Pro)

3.3 Histopathology studies

The histological sections of the liver of control and extract treated groups are represented in Figure 4. The changes in cellular morphology of hepatocytes are given in Table 4. In PCN group, the architecture of liver in portal triad was destroyed along with necrosis of periportal hepatocytes and destruction of bile duct and blood vessels. There was also moderate to severe fatty change, plenty of chronic inflammatory infiltrate in portal triad. In LIV52 treated group the architecture of liver was normal, minimal chronic inflammatory infiltrate in peripheral hepatocytes, and no fatty changes were observed. The pretreatment with sample extracts helped in restoring the hepatic architecture near to the standard drug treatment, with minimal damage when compared to the PCN group. In all the experimental groups, necrosis in portal triad with chronic inflammatory infiltration with occasional foci was observed along with mild fatty change in peripheral hepatocytes, however in ABM80 group there was mild to moderate fatty change in peripheral hepatocytes.



CON-Control, PCN-Positive Control, LIV52-Livosin 52, ALAE-leaf aqueous ext, ALM80-leaf 80% MeOH ext, ABAE-bark aqueous ext, ABM80-bark 80% MeOH ext

Figure 4: Changes in histopathological in liver of control and extract treated groups

4. Discussion

Oxidative stress and the metabolic stress contribute for hepatic injury and dysfunction. Medicinal plants are blessed with a wide array of phytochemicals, most of which are potent antioxidants, and even some of the phytoconstituents from the medicinal plants have the capacity to potentiate the regeneration of the damaged hepatocytes.

Many hepatic injury models or protocols have been developed to assess the hepatoprotective efficacy of the medicinal plants, of which CCl_4 induced hepatic damage in rats/mice is most widely

accepted model system to study the protective effect of samples against oxidative damage in hepatocytes. Carbon tetrachloride (CCl_4), is a widely used and well-established hepatotoxin. Various studies have demonstrated that the liver is not the only target organ of CCl_4, it causes free radical generation in other tissues also such as kidneys, heart, lung, testis, brain and blood (Ahmed and Urooj, 2010). CCl_4 is bio-transformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturbing Ca^{2+} haemostasis and finally resulting in cell death (Kanaujia *et al.*, 2011; Raju and Sreekanth, 2010). The extent of toxic effect on normal liver functioning can be estimated by the activities of serum marker enzymes, like AST, ALT, ALP and by the histopathological observation. The tendency of the aforementioned enzymes to return to near normal levels in extract administered group is a clear manifestation of hepatoprotective effect of the extracts.

In the present study, reduction in the elevated levels of AST and ALT towards the normal range was observed in sample treated groups, when compared to PCN group, indicating hepatoprotective effect. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function in damaged hepatocytes due CCl_4 toxicity. Hence, all the extracts significantly ($p<0.05$) blunted the increased activities of these enzymes and the level of bilirubin in the serum, showing the hepatoprotective effect.

Research studies have shown that drugs having antioxidant activity are protective against CCl_4 induced hepatotoxicity (Roy *et al.*, 2006). The inhibition of free radical generation is important in the protection against CCl_4 induced hepatic injury (Bhattacharya *et al.*, 2003). Initial phytochemical screening of *A.altilis* leaf and bark samples showed the presence of flavonoids, saponins, alkaloids, polyphenols and other phytoconstituents. Literature indicates that 160 phytoconstituents from 101 plant families have anti-hepatotoxic activity (Tapas *et al.*, 2008). Of these plant metabolites, the phenolic components such as flavonoids (Nijveldt *et al.*, 2011), polyphenols (Perron *et al.*, 2009), saponins (Francis *et al.*, 2002), alkaloids, terpenoids (Thoppil and Bishayee, 2011) are known to reduce oxidative stress by virtue of their antioxidant and anti-inflammatory activities.

5. Conclusion

From the observations, it can be inferred that the presence of the above antioxidant phytochemicals in the leaves and bark of *Artocarpus altilis* may act to reduce the CCl_4 induced oxidative stress, in turn reducing the generation of free radicals due ionization of CCl_4. Hence, there is potential to utilize the plant in functional food formulations, as a neutraceutical and phytomedicine, to minimize oxidative stress.

Acknowledgement

The authors acknowledge Special Assistance Programme (Phase-I) University Grant Commission, New Delhi, India, for financial assistance.

Conflict of interest

We declare that we have no conflict of interest.

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