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Evaluation of hepatoprotective property of methanolic extract of *Alternanthera sessilis* in CCl_4 induced hepatotoxicity in rats

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Abstract
A study was carried out to evaluate the hepatoprotective property of *Alternanthera sessilis* in CCl_4 induced hepatotoxicity in rats. A total of 36 Wister rats were procured for the purpose of study, allotted into six different groups, having six numbers of animals in each. Group T1 was kept as negative control and fed standard basal diet for a period of four weeks. Group T2 was kept as positive control and subjected to CCl_4 plus liquid paraffin (50% v/v) treatment at the rate of 2ml/kg body weight twice a week for three weeks. Group T3 was treated with Silymarin at 100 mg/kg body weight concurrently with CCl_4 toxicity for four weeks. Group T4, T5 and T6 were treated with methanolic extract of *Alternanthera sessilis* at 100 mg/kg body weight, 300 mg/kg body weight and 900 mg/kg body weight respectively and concomitantly with CCl_4 toxicity. Results revealed that there was significant decrease in the in the various hepatic biomarkers viz. ALT, AST, ALP, bilirubin in the methanolic extract of *Alternanthera sessilis* treated groups T4, T5, T6 as compared to the positive control group T2. Histopathological examination also showed that the hepatic damage induced by CCl_4 toxicity was significantly reduced in the methanolic extract of *Alternanthera sessilis* treated groups. Thus it can be inferred that methanolic extract of *Alternanthera sessilis* has significant therapeutic effect on liver.

Keywords: *Alternanthera sessilis*, CCl_4, hepatic biomarkers, hepatotoxicity, methanolic extract

Introduction
Liver is a very vital organ which performs a central role in metabolic homeostasis (Taub, 2004) [14]. The liver is not only entrusted with performing physiological functions but is also responsible to provide protection against the hazards of harmful drugs and chemicals (Palanivel et. al 2008) [9]. The liver happens to be the primary site of contact for a wide variety of orally ingested therapeutic drugs, alcohol and other xenobiotics from intestinal absorption, thus making this organ particularly susceptible to chemical-induced injury (Gu and Manautou, 2012) [6]. A significantly wide spectrum of liver pathologies ranging from necrosis to cancer has been observed following chemical exposures both in humans and in animal models (Mir *et al.*, 2010) [8]. Despite rapid advances being made in the medical science, yet the availability of effective hepatoprotective drugs seems elusive and the need for alternatives in the treatment of liver disease has only deepened in the recent years. Alternative and complementary medicine holds tremendous promise as a remedy for various liver diseases and is very different from western or allopathic medicine (Al- Zahim *et al.*, 2013) [1]. Plants have been utilized for its therapeutic efficacy in the treatment of various liver diseases since time immemorial (Wahlang *et al.*, 2013) [15]. *Alternanthera sessilis* is a perennial prostate herb which is known to possess significant anti- inflammatory (Subhashini et. al, 2010) [12] anti-oxidant and free radical scavenging properties (Borah *et al.*, 2011) [3]. In view of the need to reduce the cost of treatment and also do away with the various side effects of allopathic treatment, the present study has been undertaken to evaluate the efficacy of *Alternanthera sessilis* as a hepatoprotective agent.

Materials and Methods
Ethical approval
The experiment was conducted in accordance with the guidelines laid down by the Institutional Animal Ethics Committee bearing approval no. 770/ac/CPCSEA/FVSc/AAU/IAEC/15-16/354 dated 10.04.2015.

Experimental design

A study was undertaken in the department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati, Assam to evaluate the hepatoprotective property of *Alternanthera sessilis* in CCl_4 induced hepatotoxicity in rats. A total of 36 Wister rats were procured for the purpose of study, allotted into six different groups, having six numbers of animals in each. Group T1 was kept as negative control and fed standard basal diet for a period of four weeks. Group T2 was kept as positive control and subjected to CCl_4 plus liquid paraffin (50% v/v) treatment at the rate of 2ml/kg body weight twice a week for three weeks. Group T3 was treated with Silymarin at 100 mg/kg body weight concurrently with CCl_4 toxicity for four weeks. Group T4, T5 and T6 were treated with methanolic extract of *Alternanthera sessilis* at 100 mg/kg body weight, 300 mg/kg body weight and 900 mg/kg body weight respectively and concomitantly with CCl_4 toxicity. Phytochemical studies of *Alternanthera sessilis* were also carried out. The dosage of *Alternanthera sessilis* was determined in keeping with the OECD guideline 423.

Histopathological Examination

Histopathological examination of the liver samples from different treatment groups was also carried out after the rats were humanely sacrificed at the end of the experiment. Two way Anova was carried out using SPSS software for statistical analysis.

Results and Discussion

Phytochemical studies of *Alternanthera sessilis* were carried out using various biochemical methods. Results revealed the presence of phlobatannins, saponins, flavonoids, steroids and terpenoids. Cardiac glycosides, tannins, anthraquinones and reducing sugar were not found in the present investigation.

Aspartate aminotransferase

Results revealed a significant decline in the serum AST levels in the Silymarin and methanonic extract treated groups as compared to the positive control group. At the end of 4th week, the AST level was recorded to be 128.09 U/L in the methanolic extract of *A. sessilis* treated group T6 which is significantly lower as compared to the positive control group T2 (175.45 U/L). Terpenoids, which was found to be present in *Alternanthera sessilis*, possesses significant hepatoprotective property (Krishnamurthy *et al.*, 2010) [7]. The decline in the Aspartate aminotransferase may be attributed to the anti-oxidant and free radical scavenging activity of terpenoids (Anil and Suresh, 2011) [2] which play a significant role in staving off the deleterious effects of oxidative stress (Brai *et al.*, 2014) [4].

Alanine aminotransferase

Results revealed that the ALT levels were significantly lower in the Silymarin and the methanolic extract treated groups as compared to the positive control group. At the end of 4th week, the ALT level was recorded to be 89.57 U/L in the methanolic extract treated group T6 which is significantly lower as compared to positive control group T2 (155.89 U/L).The flavonoids are instrumental in assuaging the damage wrought by lipid peroxidation through their anti-oxidant property (Chaudhari and Mahajan, 2016) [5]. Presence of flavonoids in *Alternanthera sessilis* may be one of the factors participating in the ameliorative counterbalance of the oxidative stress as indicated by a decline in the serum alanine aminotransferase levels.

Alkaline phosphatase

The ALP levels were found to be significantly lower in the Silymarin and methanolic extract treated groups as compared to the positive control group. At the end of 4th week, the ALP level in the positive control group T2 was recorded to be 77.61 U/L which was significantly reduced to 59.66 U/L in the methanolic extract treated group T6. Saponin, another active ingredient of *Alternanthera sessilis*, is also known to possess significant hepatoprotective property (Smith and Adanlawo, 2015) [11]. The decline in the serum alkaline phosphatase levels, suggestive of improvement in the liver condition, may be attributed to the anti- inflammatory property of saponin (Sur *et al.*, 2001) [13] which might have played a significant role in offsetting the damage caused by administration of CCl_4.

Bilirubin

A significant decrease in the serum bilirubin levels was observed in the Silymarin and methanolic extract treated groups as compared to the positive control group T2. At the end of 4th week, a significant decline in the bilirubin level from 1.94 mg/dl in the positive control group T2 to 1.24 mg/dl in the methanolic extract of *Alternanthera sessilis* treated group T6.

The hepatoprotective effect of the methanolic extract of Alternantherasessilis may be due to the synergistic effect of saponin and steroids, both of which have been known to possess significant anti- inflammatory property (Patel and Savjani, 2015) [10].

Table 1: Effect of methanolic extract of *A. sessilis* and Silymarin on Aspartate aminotransferase (U/L) in the CCl_4 induced hepatic damage in rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	$95.19^{j} \pm 0.83$	$95.02^{j} \pm 0.78$	$94.92^{j} \pm 1.12$	$95.68^{j} \pm 0.93$	$95.88^{j} \pm 0.75$	$95.34^{a} \pm 0.38$
Group T2	$95.04^{j} \pm 0.91$	$105.47^{hi} \pm 1.38$	$144.54^{b} \pm 1.59$	$173.86^{a} \pm 2.03$	$175.45^{a} \pm 1.85$	$138.87^{f} \pm 6.27$
Group T3	$95.06^{j} \pm 0.62$	$98.98^{ij} \pm 0.65$	$111.96^{gh} \pm 1.42$	$117.99^{fg} \pm 1.03$	$119.18^{f} \pm 1.24$	$108.63^{b} \pm 1.88$
Group T4	$95.32^{j} \pm 0.83$	$100.05^{ij} \pm 0.71$	$129.94^{d} \pm 1.59$	$138.31^{bc} \pm 1.89$	$139.74^{bc} \pm 1.68$	$120.67^{e} \pm 3.60$
Group T5	$95.39^{j} \pm 0.82$	$99.22^{ij} \pm 0.52$	$121.28^{ef} \pm 1.25$	$132.97^{cd} \pm 1.33$	$132.89^{cd} \pm 1.44$	$116.35^{d} \pm 3.04$
Group T6	$95.29^{j} \pm 0.89$	$99.04^{ij} \pm 0.60$	$118.74^{fg} \pm 1.33$	$127.08^{de} \pm 1.56$	$128.09^{de} \pm 1.37$	$113.65^{c} \pm 2.63$
Total	$95.21^{a} \pm 0.31$	$99.63^{b} \pm 0.60$	$120.23^{c} \pm 2.63$	$130.98^{d} \pm 4.02$	$131.87^{d} \pm 4.08$	115.59 ± 1.70

Table 2: Effect of methanolic extract of *A. sessilis* and Silymarin on Alanine Aminotransferase (U/L) in the CCl_4 induced hepatic damage in rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	$67.92^{j} \pm 1.26$	$66.53^{j} \pm 1.01$	$67.10^{j} \pm 1.21$	$66.40^{j} \pm 1.05$	$66.31^{j} \pm 0.61$	$66.85^{a} \pm 0.45$
Group T2	$66.75^{j} \pm 1.53$	$71.06^{ij} \pm 0.69$	$92.87^{cd} \pm 1.43$	$151.58^{a} \pm 1.91$	$155.89^{a} \pm 1.11$	$107.63^{f} \pm 1.21$
Group T3	$66.41^{j} \pm 1.36$	$67.13^{j} \pm 0.62$	$74.88^{hi} \pm 1.02$	$82.83^{fg} \pm 1.80$	$83.11^{fg} \pm 1.14$	$74.87^{b} \pm 1.44$

Group T4	66.31^{j} ± 1.12	67.11^{j} ± 0.73	86.01^{ef} ± 0.98	96.37^{bc} ± 1.15	100.73^{b} ± 1.33	83.31^{d} ± 2.71
Group T5	66.07^{j} ± 1.04	67.17^{j} ± 0.99	82.67^{fg} ± 0.85	90.75^{cde} ± 1.16	93.47^{cd} ± 1.25	80.03^{c} ± 2.18
Group T6	66.08^{j} ± 0.83	67.22^{j} ± 0.97	79.53^{gh} ± 1.07	87.77^{def} ± 1.04	89.57^{de} ± 1.04	78.04^{c} ± 1.88
Total	66.59^{a} ± 0.47	67.70^{b} ± 0.41	80.51^{c} ± 1.44	95.95^{d} ± 4.52	98.18^{e} ± 4.74	81.79 ± 1.67

Table 3: Effect of methanolic extract of *A. sessilis* and Silymarin on Alkaline Phosphatase (U/L) in the CCl_4 induced hepatic damage in rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	48.55^{jk} ± 0.67	47.01^{k} ± 1.14	47.64^{jk} ± 0.69	47.30^{jk} ± 1.18	46.88^{k} ± 0.93	47.47^{a} ± 0.41
Group T2	48.96^{ijk} ± 0.67	54.25^{ghij} ± 0.92	70.40^{bc} ± 2.43	75.51^{ab} ± 1.88	77.61^{a} ± 1.06	65.34^{d} ± 2.24
Group T3	48.56^{jk} ± 0.93	50.71^{hijk} ± 0.91	55.61^{fghi} ± 1.47	55.66^{fghi} ± 1.23	56.52^{defgh} ± 0.94	53.41^{b} ± 0.75
Group T4	48.36^{jk} ± 1.38	50.82^{hijk} ± 0.43	57.99^{defg} ± 1.82	63.48^{cd} ± 1.34	63.12^{de} ± 1.09	56.75^{c} ± 1.27
Group T5	47.95^{jk} ± 1.37	50.21^{hijk} ± 0.81	56.38^{efgh} ± 1.65	61.85^{def} ± 1.57	60.93^{defg} ± 1.15	55.46b^{c} ± 1.18
Group T6	47.89^{jk} ± 1.11	50.08^{hijk} ± 0.77	56.01^{fgh} ± 1.07	58.76^{defg} ± 2.46	59.66^{defg} ± 1.04	54.48b^{c} ± 1.05
Total	48.38^{a} ± 0.41	50.51^{b} ± 0.48	57.34^{c} ± 1.29	60.43^{d} ± 1.57	60.78^{d} ± 1.59	55.49 ± 0.65

Table 4: Effect of methanolic extract of *A. sessilis* and Silymarin on Bilirubin level (mg/dl) in the CCl_4 induced hepatic damage in rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	0.87^{mno} ± 0.01	0.87^{mno} ± 0.01	0.87^{mno} ± 0.01	0.87^{mno} ± 0.01	0.85^{mno} ± 0.01	0.87^{a} ± 0.00
Group T2	0.89^{mno} ± 0.01	0.92^{klmn} ± 0.01	1.10^{ghi} ± 0.02	1.64^{b} ± 0.02	1.94^{a} ± 0.04	1.30^{f} ± 0.08
Group T3	0.86^{mno} ± 0.01	0.89^{mno} ± 0.01	0.89^{mno} ± 0.01	1.06^{hij} ± 0.02	1.17^{fg} ± 0.02	0.97^{b} ± 0.02
Group T4	0.84^{no} ± 0.01	0.88^{mno} ± 0.01	1.01^{ijk} ± 0.02	1.31^{de} ± 0.02	1.51^{c} ± 0.03	1.11^{e} ± 0.05
Group T5	0.82^{o} ± 0.01	0.88^{mno} ± 0.01	0.98^{jkl} ± 0.01	1.18^{fg} ± 0.02	1.38^{d} ± 0.02	1.05^{d} ± 0.04
Group T6	0.86^{mno} ± 0.01	0.89^{mno} ± 0.01	0.95^{klm} ± 0.01	1.13^{gh} ± 0.02	1.24^{ef} ± 0.02	1.01^{c} ± 0.03
Total	0.86a ± 0.00	0.89^{b} ± 0.00	0.96^{c} ± 0.01	1.20^{d} ± 0.04	1.35^{e} ± 0.06	1.05 ± 0.02

Histopathology

Histopathological examination revealed that that there was massive fatty change with perilobular necrosis in the CCl_4 treated positive control group (Figure 1). In the silymarin treated group, minor fatty changes were found along with centri-lobular regeneration (Figure 2). In the group treated with methanolic extract of *Alternanthera sessilis* at the rate of 100 mg.kg^{-1}, moderate fatty changes with slight necrosis were observed (Figure 3). In the group treated with methanolic extract of *Alternanthera sessilis* at the rate of 300 mg.kg^{-1}, moderate fatty changes were observed along with the presence of hyperchromic nuclei indication regeneration of hepatocytes in the CCl4 induced liver damage (Figure 4). In the group treated with methanolic extract of *Alternanthera sessilis* at the rate of 900mg.kg^{-1}, it revealed moderate fatty changes along with the presence of hyperchromic nuclei in the CCl_4 induced liver damage (Figure 5).

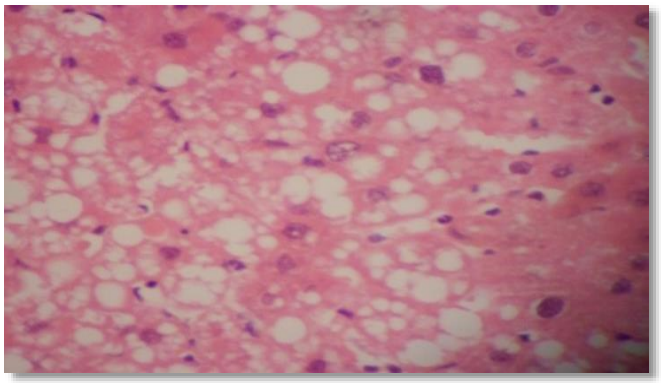

Fig 1

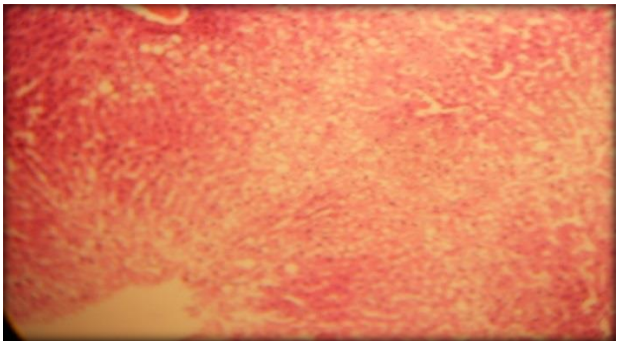

Fig 2

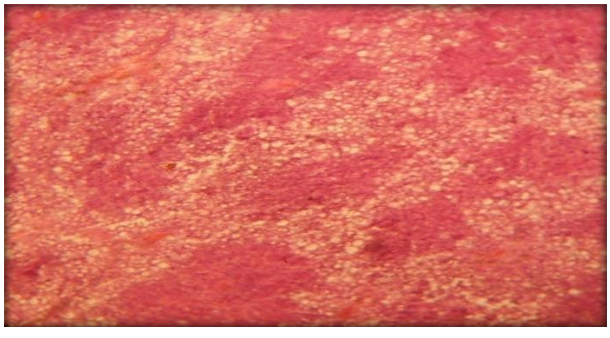

Fig 3

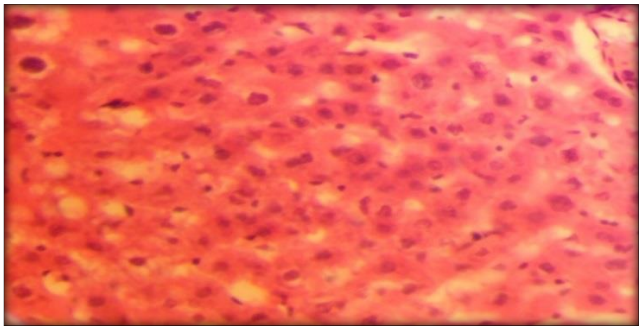

Fig 4

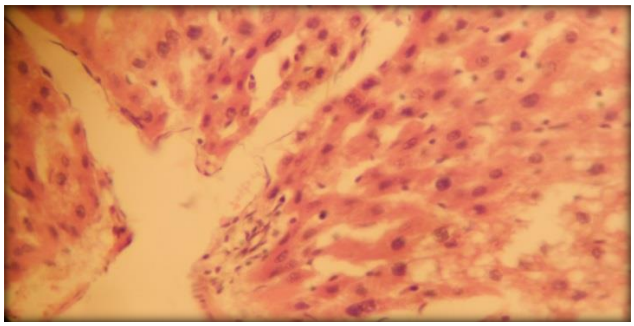

Fig 5

Acknowledgement
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