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Evaluation of Hepatoprotective Property of *Alternanthera sessilis* in CCl_4 Induced Hepatotoxicity in Rats

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A B S T R A C T

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 selected for the purpose of study, segregated 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 aqueous 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 various hepatic biomarkers viz. ALT, ALP, AST in the aqueous extract of *Alternanthera sessilis* treated groups T4, T5 and T6 as compared to positive control group T2. Histopathological examination also revealed that the hepatic damage wrought by CCl_4 toxicity was significantly thwarted in the aqueous extract of *Alternanthera sessilis* treated groups. Thus it can be inferred that aqueous extract of *Alternanthera sessilis* can significantly ameliorate signs of hepatotoxicity in the CCl_4 induced hepatotoxic rats.

Introduction

Liver is a vital and major metabolic organ repeatedly exposed to various drugs, chemicals, and toxins by virtue of our lifestyle activities (Kareem *et al.*, 2013). Oxidative stress is a redox disequilibrium in which the pro-oxidant/antioxidant balance is shifted in favour of the pro-oxidants (Sies, 1986). Major source of free radicals in vivo is autoxidation of flavinthiols, activity of electron transport chain, oxidases, cyclooxygenases, peroxidases etc.(Forman *et al.*, 2010). Liver diseases pose a serious challenge to international public health (Ahsan *et al.*, 2009).A vast number of medicinal plants have traditionally been used in the ayurvedic system of medicine for the treatment of liver disorders and have been scientifically proven to have hepatoprotective properties (Sharma *et al.*, 1991; Murty *et al.*, 1993). Interest in complementary and alternative medicine (CAM) is increasing throughout the world probably because there

are only few universally effective and available options for the treatment of common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis (Chevaliez and Pawlotsky, 2009; Ghani, 2009; Cubero *et al.,* 2009, Lam and Younossi, 2009; Torres and Harrison, 2008; Kashi *et al.,* 2008). Plants are natural producers of chemical substances, providing potential treatment of human ailments since ancient times (Rolf and Eickhoff, 2015). CCl_4 is a well-known hepatotoxic industial solvent (Abraham *et al.,* 1999; Guven and Gumez, 2003).

The free radicals generated from CCl_4 and the parent molecule by itself, damage endoplasmic reticulum (ER), which leads to accumulation of lipids, reduced protein synthesis and mixed function oxidases activity (Weber *et al.,* 2003). Lipid peroxidation (LPO) has been a frequently invoked mechanism in ROS-induced cell death and liver injury (Kehrer, 2008; Negre-Salvayre *et al.,* 2010, Sevanian *et al.,* 1985).

Recently, a trend has developed towards employing certain herbal medicines to manage hepatotoxicity (Khurelbat *et al.,* 2014). Herbal plants are bereft of many side effects that otherwise accompany allopathic medicines.

As more and more people are beginning to repose their faith in herbal treatment as alternative to allopathic medicines, it has rekindled interest among the scientific community to investigate further in that direction and come up with a herbal remedy which is not only safe but also possesses significant ameliorative effect on the liver. In view of the above, the present study has been undertaken to evaluate the efficacy of aqueous extract of *Alternanthera sessilis* in mitigating the CCl_4 induced liver damage in rats.

Materials and Methods

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 selected for the purpose of study, segregated 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 aqueous 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 congruence with the OECD guideline 423.

Histopathological examination of the liver samples from different treatment groups was also done 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

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 detected in the present investigation.

Aspartate aminotransferase

Results revealed that there was significant decrease in the AST levels in the Silymarin and aqueous extract treated groups as compared to the positive control group. At the end of 4th week, the AST level was recorded to be 127.30 U/L in the aqueous extract of *A. sessilis* treated group T6 which is significantly lower as compared to the positive control group T2 (174.91 U/L) (table 1). Studies have shown that saponin, a phytoconstituent which was found to be present in *Alternanthera sessilis*, possesses significant hepatoprotective property and is also known to alter expression of hepatic oxidative stress markers and proinflammatory mediators, combined with restoring liver CYP2E1 level (Huang *et al.,* 2012).

The decrease in serum AST levels in the aqueous extract of *Alternanthera sessilis* treated groups may be attributed to the presence of saponin, which exerts its hepatoprotective effect through a multitude of ways viz. dramatic decreases of iNOS level and IL-6 level which is responsible liver damage (Liping *et al.,* 2012).

Alanine aminotransferase

The ALT levels were found to be significantly lower in the Silymarin and the aqueous extract treated groups as compared to the positive control group. At the end of 4th week the ALT level in the positive control group T2 was recorded to be 155.20 U/L which was significantly reduced to 89.02 U/L in the aqueous extract of *Alternanthera sessilis* treated group T6. (Table 2). The decrease in the ALT levels in the groups treated with aqueous extract of *Alternanthera sessilis* may be attributed to the presence of flavonoids which also have been known to possess significant hepatoprotective effect (Akachi *et al.,* 2010). A substantial body of research have strongly linked the presence of flavonoids to its hepatoprotective effect and there are several reports to show that flavonoid containing plant extracts help to stave off the hepatic damage caused by various noxious agents (Nquyen *et al.,* 2017; Wu *et al.,* 2006).

Alkaline phosphatase

A significant decrease in the ALP levels was observed in the Silymarin and aqueous extract treated groups as compared to the positive control group. At the end of 4th week, the ALP level in the aqueous extract of A. sessilis treated group T6 was recorded to be 60.03 U/L, which significantly lower than the positive control group T2 (77.13 U/L) (table3). Terpenoids, another phytoconstituent present in *Alternanthera sessilis*, have also been well documented to possess hepatoprotective property (Alqasoumi *et al.,* 2012). The hepatoprotection conferred through treatment with aqueous extract of *Alternanthera sessilis* may be attributed to the anti-oxidant and free radical properties of terpenoids which help to neutralize the peroxy free radical generated by CCl_4 (Anil *et al.,* 2011; Borah *et al.,* 2011).

Bilirubin

Results revealed that the serum bilirubin levels were significantly reduced in the Silymarin and aqueous extract of *Alternanthera sessilis* treated groups as compared to the positive control group. At the end of 4th week, a significant decline in the bilirubin level from 1.95 mg/dl in the positive control group T2 to 1.23 mg/dl in the aqueous extract of *Alternanthera sessilis* treated group T6 was observed (table 4).The decrease in the serum bilirubin in the aqueous extract treated groups indicates that the liver damage caused by exposure to CCl_4 has been markedly thwarted.

Table.1 Effect of aqueous extract of *A. sessilis* and Silymarin on Aspartate Aminotransferase (U/L) in CCl4 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	$95.71^{i} \pm 0.71$	$95.82^{i} \pm 0.74$	$95.04^{i} \pm 0.77$	$95.64^{i} \pm 0.82$	$95.13^{i} \pm 0.59$	$95.47^{a} \pm 0.31$
Group T2	$95.48^{i} \pm 0.73$	$105.21^{h} \pm 1.12$	$143.34^{b} \pm 1.47$	$170.88^{a} \pm 1.42$	$174.91^{a} \pm 1.40$	$137.96^{f} \pm 6.10$
Group T3	$95.26^{i} \pm 1.05$	$98.01^{i} \pm 1.26$	$109.25^{g} \pm 1.75$	$116.65^{f} \pm 1.25$	$119.26^{ef} \pm 1.55$	$107.69^{b} \pm 1.88$
Group T4	$95.87^{i} \pm 1.07$	99.79h$^{i} \pm 1.07$	$128.39^{c} \pm 1.20$	$137.66^{b} \pm 1.35$	$139.47^{b} \pm 1.66$	$120.23^{e} \pm 3.52$
Group T5	95.94i ± 0.86	$98.71^{i} \pm 0.63$	$120.89^{def} \pm 1.43$	$129.23^{c} \pm 1.65$	$130.65^{c} \pm 1.40$	$115.09^{d} \pm 2.82$
Group T6	$95.52^{i} \pm 0.87$	$98.00^{i} \pm 0.92$	$117.93^{f} \pm 1.04$	$125.59^{cde} \pm 1.23$	$127.30^{cd} \pm 1.22$	$112.87^{c} \pm 2.55$
Total	$95.63^{a} \pm 0.34$	$99.26^{b} \pm 0.62$	$119.14^{c} \pm 2.59$	$129.28^{d} \pm 3.88$	$131.12^{d} \pm 4.08$	114.88 ± 1.66

Table.2 Effect of aqueous extract of *A. sessilis* and Silymarin on Alanine Aminotransferase (U/L) level in CCl_4 induced liver damage in rats

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	$66.51^{j} \pm 1.00$	$66.27^{j} \pm 0.94$	$66.29^{j} \pm 0.66$	$66.44^{j} \pm 0.68$	$66.47^{j} \pm 0.96$	$66.40^{a} \pm 0.36$
Group T2	$66.11^{j} \pm 1.08$	$70.82^{ij} \pm 0.83$	$92.06^{cd} \pm 1.11$	$151.92^{a} \pm 1.36$	$155.30^{a} \pm 1.33$	$107.24^{f} \pm 7.23$
Group T3	$66.28^{j} \pm 0.60$	$67.47^{j} \pm 0.99$	$74.59^{hi} \pm 0.97$	$82.02^{fg} \pm 1.05$	$82.81^{fg} \pm 1.21$	$74.63^{b} \pm 1.36$
Group T4	$66.13^{j} \pm 0.82$	$67.09^{j} \pm 1.17$	$86.01^{ef} \pm 0.87$	$95.91^{bc} \pm 1.16$	$99.32^{b} \pm 0.79$	$82.89^{e} \pm 2.63$
Group T5	$66.21^{j} \pm 0.86$	$67.14^{j} \pm 1.16$	$81.11^{fg} \pm 1.50$	$88.58^{de} \pm 0.98$	$91.83^{cd} \pm 0.78$	$78.97^{d} \pm 2.03$
Group T6	$66.13^{j} \pm 0.92$	$67.05^{j} \pm 0.86$	$78.08^{gh} \pm 0.97$	$85.18^{ef} \pm 1.20$	89.02d$^{e} \pm 0.99$	$77.09^{c} \pm 1.77$
Total	$66.23^{a} \pm 0.34$	$67.64^{a} \pm 0.45$	$79.69^{b} \pm 1.44$	$95.01^{c} \pm 4.58$	$97.46^{e} \pm 4.71$	81.20 ± 1.66

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

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Aggregate
	Mean	Mean	Mean	Mean	Mean	Mean
Group T1	48.28^{gh} ± 1.18	47.02^{h} ± 1.00	47.31^{h} ± 0.73	46.67^{h} ± 1.15	46.58^{h} ± 0.76	47.17^{a} ± 0.42
Group T2	48.10^{gh} ± 1.07	54.18^{efg} ± 0.96	69.06^{b} ± 1.27	75.46^{a} ± 1.07	77.13^{a} ± 1.49	64.78^{d} ± 2.21
Group T3	48.10^{gh} ± 1.33	50.41^{fgh} ± 0.94	54.01^{efg} ± 1.46	55.43^{def} ± 1.09	57.77^{cde} ± 1.12	53.14^{b} ± 0.82
Group T4	48.05^{gh} ± 1.17	50.44^{fgh} ± 0.54	57.23^{cde} ± 0.91	63.24^{bc} ± 1.43	63.17^{bc} ± 1.17	56.43^{c} ± 1.25
Group T5	48.00^{gh} ± 1.10	50.43^{fgh} ± 0.56	56.25^{def} ± 1.04	61.09^{cd} ± 1.27	61.02^{cd} ± 1.12	55.36^{c} ± 1.09
Group T6	48.24^{gh} ± 1.18	50.37^{fgh} ± 0.44	55.89^{def} ± 1.27	57.43^{cde} ± 1.62	60.03^{cde} ± 0.64	54.39^{bc} ± 0.94
Total	48.13^{a} ± 0.44	50.48^{b} ± 0.46	56.63^{c} ± 1.17	59.89^{d} ± 1.55	60.95^{d} ± 1.57	55.21 ± 0.64

Table.4 Effect of aqueous extract of *A.sessilis* and Silymarin on bilirubin level (mg/dl) in CCl_4 induced liver 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^{i} ± 0.01	0.87^{i} ± 0.01	0.87^{i} ± 0.01	0.85^{i} ± 0.01	0.86^{i} ± 0.01	0.86^{a} ± 0.00
Group T2	0.85^{i} ± 0.01	0.90^{hi} ± 0.01	1.06^{fg} ±0.02	1.65^{b} ± 0.02	1.95^{a} ± 0.03	1.28^{e} ± 0.08
Group T3	0.86^{i} ± 0.02	0.88^{hi} ± 0.01	0.90^{hi} ± 0.01	1.04^{fg} ± 0.01	1.07^{ef} ± 0.02	0.95^{b} ± 0.02
Group T4	0.85^{i} ± 0.01	0.88^{hi} ± 0.01	0.97^{gh} ± 0.01	1.17^{cd} ± 0.02	1.23^{c} ± 0.02	1.02^{d} ± 0.03
Group T5	0.87^{i} ± 0.01	0.88^{hi} ± 0.01	0.93^{hi} ± 0.01	1.10^{def} ± 0.03	1.15^{cde} ± 0.02	0.99^{c} ± 0.02
Group T6	0.87^{i} ± 0.02	0.89^{hi} ± 0.01	0.92^{hi} ± 0.01	1.08^{def} ± 0.02	1.11^{def} ± 0.03	0.97^{bc} ± 0.02
Total	0.86^{a} ± 0.01	0.88^{a} ± 0.00	0.94^{b} ± 0.01	1.15^{c} ± 0.04	1.23^{d} ±0.06	1.01 ± 0.02

Fig.1

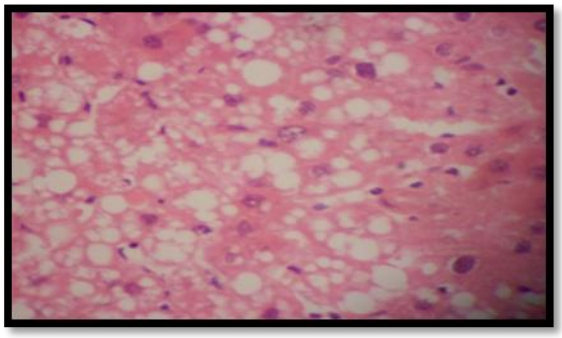


Fig.2

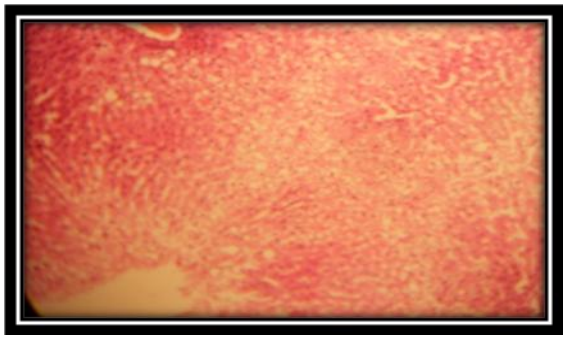


Fig.3

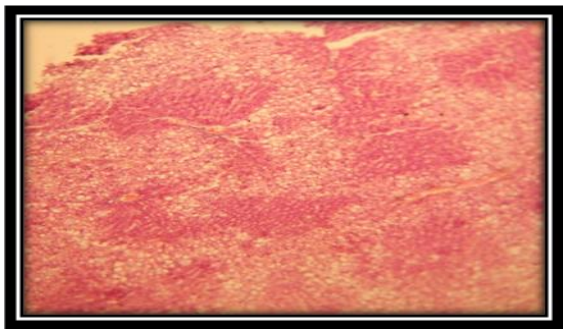


Fig.4

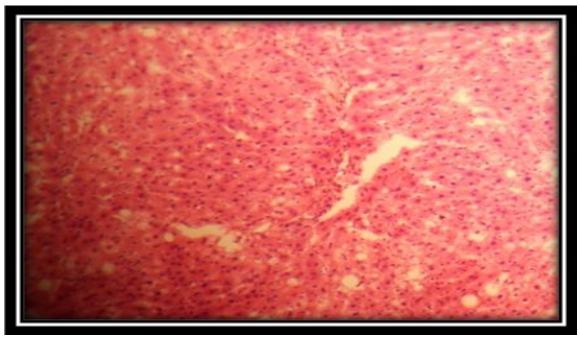
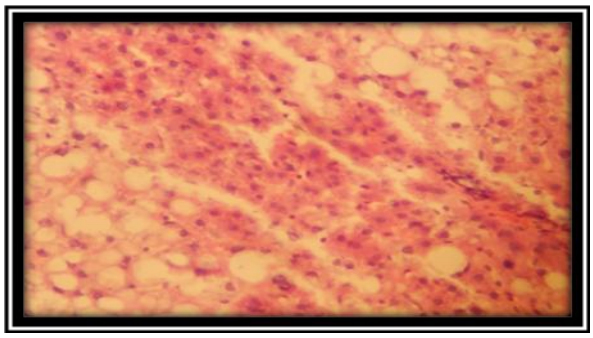


Fig.5



This may be attributed to the presence of a confluence of phytoconstituents viz. Terpenoids, flavonoids saponin etc which are known to exert hepatoprotective effect through its anti-oxidant and free radical scavenging activity.

Histopathology

Histopathological examination revealed that in the CCl_4 treated positive control group, there was massive fatty change with perilobular necrosis (Figure 1). In the silymarin treated group, slight fatty changes with centri- lobular regeneration were observed (Figure 2). In the group treated with aqueous extracts of *Alternanthera sessilis* at the rate of 100mg.kg^{-1}, moderate fatty changes were observed with necrosis (Figure 3). In the group treated with aqueous extract of *Alternanthera sessilis* at the rate of 300mg.kg^{-1}, it revealed moderate fatty change, there was also presence of hyperchromic nuclei indicating regeneration of hepatocytes in CCl_4 induced liver damage (Figure 4). In the group treated with aqueous extract of *Alternanthera sessilis* at the rate of 900mg.kg^{-1}, histopathological examination revealed moderate fatty changes with similar hyperchromic nuclei in ccl_4 induced liver damage (Figure 5)

The results clearly show that administration of aqueous extract of *Alternanthera sessilis* across varying dose range has significantly brought down the hepatic enzyme levels in the blood which is further underscored by histopathological examination showing significant regenerative changes in the groups treated with aqueous extract of *Alternanthera sessilis*. Thus, it can be inferred that aqueous extract of *Alternanthera sessilis* possesses significant hepatoprotective activity and is a potential candidate as an herbal alternative against various liver ailments.

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