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Evaluation of Hepatoprotective and antioxidant activity of *Sauropus bacciformis* whole plant methanolic extracts against CCL_4: Induced liver injury in rats

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Abstract
This study was designed to evaluate the hepatoprotective and antioxidant effect of methanol extract of whole plant of *Sauropus bacciformis* on CCl_4 induced hepatotoxicity in rats. Activities of liver marker enzymes, SGOT, SGPT and ALP, total protein, albumin, globulin, total, conjugated and unconjugated bilirubins at an oral dose of methanol extract of *Sauropus bacciformis* (150 and 300mg/kg) showed a significant hepatoprotective effect. Regarding antioxidant activity, methanol extract of *Sauropus bacciformis* exhibited a significant effect showing increasing levels of SOD, CAT, GPx, GSH and GRD by reducing malondialdehyde (MDA) levels.

Keywords: hepatoprotective activity, antioxidant, CCl4, bilirubin

1. Introduction
Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effect. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity [1]. Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases [2]. Silymarin extract from the seeds of the plant *Silybum marianum*, also called milk thistle. It has been described to be an antioxidant and exhibits anticarcinogenic, antiinflammatory, hepatoprotection and growth modulatory effects [3, 4]. In this study, silymarin was used as a positive control to against the paracetamol-induced acute hepatic damage in rats. Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids [5] and Vitamin C [6] etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [7]. These has been a growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research in to their potential benefits to human health. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degeneration diseases [8]. Sauropus bacciformis (L.) Airy Shaw is a herb growing in seashore sandy tracts, especially in brackish clayey soil near sea level to below 100 m. *Sauropus bacciformis* is commonly called Kuruvi Thengai, Thengai Keerai and it is used by the rural folk for medicinal purpose. The aerial parts of the plant is used against gastrointestinal problems. The plant paste is given with Piper betel orally (9). They use the aerial parts as a green vegetable. The plant is also used by the local people for skin diseases. Realizing the fact, this research was carried out to evaluate the antioxidant and hepatoprotective activity of *S. bacciformis* whole plant extract against CCL_4-induced hepatic damage in rats.

2. Materials and methods
Plant material
The well grown whole plant of *Sauropus bacciformis* was collected from coastal regions of Thoothukudi, District, Tamil Nadu.With the help of local flora,voucher specimens w ere identified and preserved inthe Research Department of Botany, St. Mary's College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract for phytochemical screening and Hepatoprotective studies
The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder,which was then subjected to extraction in a Soxhlet apparatus using methanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (10, 11). The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extract were used for hepatoprotective studies.

Animals
Normal healthy male Wistar albino rats (180-240gm) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum.*

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 150,and 300 mg/kg body weight.

Experimental Design

In the investigation, a total of 30 rats (25 CCl4 hepatictoxicity induced rats and 6 normal rats) were taken and divided into five groups of 6 rats each.

Group I: Rats received normal saline was served as a normal control.

Group II: CCl4 hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl4 for 14 days.

Group III: Liver injured rats received methanol extract of whole plant of *Sauropus bacciformis* at the dose of 150 mg/kg body weight for 14 days.

Group IV: Liver injured rats received methanol extract of whole plant of *Sauropus bacciformis* at the dose of 300mg/kg body weight for 14 days.

Group V: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

Biochemical Analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000kg for 10 minutes. Serum protein (12) and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of (13). Serum alkaline phosphatase (ALP) was measured by the method of (14).Total bilirubin and conjugated bilirubin were determined as described by (15). The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gammanglutamyl transpeptidase (GGTP) was estimated by the method of (16). Liver homogenates (10%W/V) were prepared in ice cold10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa. Enzymatic antioxidants, superoxide dismutase (SOD) (17)Catalase (18,19) and non enzymatic antioxidant glutathione peroxidase (GPx) (20) glutathione reductase (GRD) (21) and reduced glutathione (GSH) (22)were also assayed in liver homogenates.

Statistical Analysis

The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. $p<0.001$, $p<0.01$ and $p<0.05$ were considered as statistical significance using SPSS Software.

3. Results

The methanol extract of whole plant of *Sauropus bacciformis* subjected for phytochemical study showed the presence of alkaloids, anthroquinons, quinones, catachins, coumarins, terpenoids, sugars, glycosides, flavonoids, saponins, steroids, phenols, and tannins. The methanol extract did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose. The effect of methanol extract of *Sauropus bacciformis* on body weight of the normal, CCl4 intoxicated and drug treated rats are shown in Table 1.

Table 1: Effect of *Sauropus bacciformis* effect on the body weight of in the normal, liver damaged and drug treated rats

Treatment	Dose	Initial Body Weight(g)	Final Body Weight(g)	Mean Weight Gain (G↑)/Loss(L↓)	Difference %
Group 1	0.9%Saline	158.84±4.39	188.36±3.93	29.52↑	18.58
Group 11	0.9% Saline	193.55±3.86	171.65±4.30	21.90↓	11.31
Group 111	150(mg/kg)	186.39±4.85	181.38±3.74^{na}	5.01↓	2.61
Group 1V	300(mg/kg)	187.87±5.36	194.16±5.63^{*aa}	6.29↑	3.20
Group V	100(mg/kg)	187.25±4.34	193.54±4.88^{nsa}	8.29↑	4.40

Values are mean± SD of 6 animals in each group. Staistical analysis ANOVA followed by Dunnett t-test. $^{*}P<0.05$;$^{**}P<0.01$ as compared with Normal Control to liver damaged control NS: not significant.

Table 2 shows the effect of methanol extract of *Sauropus bacciformis* on serum total protein, albumin, globulin, A/G ratio, serum transaminases, Alkaline phosphatases in CCl4 intoxicated rats.

Table 2: Effect of *Sauropus bacciformis* effect on the serum protein, albumin, globulin concentration and serum GOT, GPT and ALP enzyme activity in the normal, liver damaged and drug treated rats

Groups	Dose	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group 1	0.9%Saline	8.58±1.65	4.93±0.17	3.65±0.12	1.3:1	12.63±0.58	19.46±0.27	181.63±4.23
Group 11	0.9% Saline	6.93±0.93*	4.11±0.34	2.82±0.11*	1.4:1	43.92±0.63**	53.63±0.38**	248.74±6.85**
Group 111	150(mg/kg)	6.14±0.23*	3.96±0.16*	2.18±0.52	1.8:1	36.93±0.88*	31.54±0.67^{*a}	209.16±5.39^{naa}
Group 1V	300(mg/kg)	8.10±0.46^{ns}	4.63±0.23	3.47±0.72	1.3:1	21.68±0.28^{*aa}	28.93±0.34^{a}	186.27±4.84^{aa}
Group V	100(mg/kg)	8.36±0.38^{aa}	4.71±0.11	3.65±0.18	1.3:1	13.99±0.18^{aa}	18.26±0.22^{aa}	173.16±2.65^{aa}

Values are mean ± SD of 6 animals in each group. Staistical analysis ANOVA followed by Dunnett t-test. *P<0.05;**P<0.01 as compared with Normal Control to liver damaged control NS: not significant.

There was a significant (p< 0.01) increase in serum GOT,GPT and ALP levels in CCl4 intoxicated group (Group II)compared to the normal control group (Group I). The total protein and albumin levels were significantly (p< 0.01) decreased to 6.93g/dl and 4.11g/dl in CCl4 intoxicated rats from the levels of 8.58g/dl and 4.93g/dl respectively in normal group. Methanol extract of *Sauropus bacciformis* at the dose of 300mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level. The effect of methanol extract of *Sauropus bacciformis* on total, conjugated and unconjugated bilirubin is shown in Table 3.

Table 3: Effect of *Sauropus bacciformis* effect on the serum Total, conjugated, unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats

Treatment	Dose	Total Bilirubin (mg/dl)	Conjugated (mg/dl)	Unconjugated (mg/dl)	GGTP (U/L)
Group 1	0.9%Saline	0.84±0.12	0.28±0.17	0.56±0.14	6.23±0.94
Group 11	0.9% Saline	4.38±1.13**	3.65±0.94**	0.73±0.12*	21.84±1.08**
Group 111	150(mg/kg)	2.89±0.24^{*a}	2.16±0.13**	0.91±0.18	12.13±0.65^{a}
Group 1V	300(mg/kg)	2.13±0.75^{nsa}	1.63±0.21^{*a}	0.80±0.14^{a}	9.65±0.74^{aa}
Group V	100(mg/kg)	1.84±0.22aa	1.03±0.17aa	0.81±0.21	7.33±0.21aa

Values are mean± SD of 6 animals in each group. Staistical analysis ANOVA followed by Dunnett t-test. *P<0.05;**P<0.01 as compared with Normal Control to liver damaged control NS: not significant.

A significant elevation of total, conjugated, unconjugated bilirubin and γ -glutamyl transferase in the serum of CCl4 intoxicated group (Group II) when compared to normal control(Group I). The methanol extract of *Sauropus bacciformis* at the dose 150 mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and γ -glutamyl transferase were found to be greater in standard Silymarin (Group V) followed by Group II and Group I11 (Table 3).

The effects of methanol extract of *Sauropus bacciformis* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD) catalase (CAT) and reduced glutathione (GSH) activity is shown in Table 4.

Table 4: Effect of *Sauropus bacciformis* effect on the serum LPO, GPX, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats

Treatment	Dose	LPO (n mole of MDA/mg protein)	GPX (u/mg Protein)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
Group 1	0.9%Saline	2.635±0.014	4.934±0.081	0.409±0.073	0.324±0.024	3.911±0.014	31.94±0.24
Group 11	0.9% Saline	5.081±0.051**	1.253±0.024**	0.196±0.055**	0.126±0.018**	1.094±0.012**	9.63±0.18**
Group 111	150(mg/kg)	3.141±0.017*	2.138±0.076*	0.293±0.037^{*aa}	0.219±0.024^{**aa}	1.843±0.15*	18.66±0.34^{*a}
Group 1V	300(mg/kg)	2.738±0.024^{nsa}	3.626±0.084^{aa}	0.381±0.028^{aa}	0.304±0.013^{nsaa}	2.865±0.018^{nsa}	28.93±0.71^{aa}
Group V	100(mg/kg)	2.926±0.016^{aa}	4.683±0.075^{aa}	0.398±0.014^{aa}	0.343±0.079^{aa}	3.584±0.013^{aa}	27.14±0.68

Values are mean± SD of 6 animals in each group. Staistical analysis ANOVA followed by Dunnett t-test. *P<0.05;**P<0.01 as compared with Normal Control to liver damaged control NS: not significant.

Lipid peroxidation level was significantly (p<0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p<0.01) decreased in CCl4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with methanol extract of *Sauropus bacciformis* at the doses of 150 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and reduced glutathione levels towards the normal levels in a dose dependent manner. The results are well comparable with Silymarin (standard drug) treated group.

4. Discussion

Liver is largest organ and it is target for toxicity of its role in clearing and metabolizing chemicals through the process called detoxification (23). Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases (24). CCl4 produces an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures (25). The toxic metabolite, CCl4 radical is produced and further reacts with oxygen to give trichloromethyl peroxy radical. Cytochrome P_{450} is the

enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue, which leads to leakage of serum marker enzymes. It is possible that hepatocellular damage occurs when the free radicals generation exceeds the cellular radicals scavenging capacity (26). Assessment of liver toxicity was done by measuring the marker enzymes such as SGOT, SGPT and ALP, which are originally present in high concentration in the cytoplasm. When there is hepatic injury these enzymes leak into blood stream inconformity with extent of hepatotoxicity treatment with methanol extracts of *Sauropus bacciformis* restored the elevated levels of serum marker enzymes. The normalization of serum markers by *Sauropus bacciformis* whole plant suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl4 induced leakages of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum protein. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. The reduction in the serum albumin and globulin levels in CCl4 intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthetic function of the liver (27). Treatment with methanol extract of *Sauropus bacciformis* whole plant ameliorated the imbalance. Serium bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases.

Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes (28). Administration of *Sauropus bacciformis* decreases the level of bilirubin and increased the level of protein suggesting that it offered protection. γ -glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ -glutamyl transferase is generally elevated as a result of liver disease, since γ -glutamyl transferase is a hepatic microsomal enzyme. Serum γ -glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in γ -glutamyl transferase is parallel to those of amino transferases. The acute damage caused by CCl4 increassed the γ -glutamyl transferase level but the same attains the normal after *Sauropus bacciformis* treatment due to its antioxidant activity.

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions (29). Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated to the destructive process of liver injury due to acetaminophen administration. In the present study the elevations in the levels of end products of lipid peroxidation in the liver of the rat treated with CCl4 was observed. The increase in melondialdehyde (MDA) levels in liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *Sauropus bacciformis* whole plant significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection by methanol extract of *Sauropus bacciformis* whole plant due to its antioxidant effects.

Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes and brain (30). The elevation of MDA level, which is one of the end products of lipid peroxidation in the liver tissue, and the reduction in hepatic GSH levels are important indicators in CCl4 intoxicated rats. In this study, it was ascertained that MAD levels have been suppressed compared to CCl4 intoxicated group and CCl4 induced depletion of GSH was prevented.

Superoxide dismutase (SOD), a metallo protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defense system. It scavenges the superoxide anion to from hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical (31). In the present study, it was observed that the methanol extract of *Sauropus bacciformis* whole plant significantly increased the SOD activity in CCl4 intoxicated rats thereby diminished CCl4 induced oxidative damage.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found to the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (32). Therefore the reduction in the activity of these enzymes may result in the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of methanol extract of *Sauropus bacciformis* increased the activities of CAT in CCl4 induced liver damage in rats to prevent the accumulation of excessive free radical and protected the liver from CCl4 intoxication.

Glutathione peroxide (GPx) is a seleno enzyme, it protect the cells from damage due to free radicals like hydrogen and lipid peroxide (33). It catalyzes the reaction of hydroperoxidases with reduced glutathione to form glutathione disulphide and reduction.

5. Conclusion

The results of this study demonstrate that *Sauropus bacciformis* has potent hepatoprotective action upon carbon tetrachloride induced hepatic damage in rats. Our result showed that the hepatoprotective effect of *Sauropus bacciformis* whole plant may be due to its antioxidant and free radical scavenging properties. Hepatoprotective activity of *Sauropus bacciformis* whole plant may be due to the presence of tannins, terpenoids, flavonoids, alkaloids, saponins and phenols. *Sauropus bacciformis* whole plant methanolic extract has contributed to the reduction of oxidative stress and

showed hepatoprotective activity in experimental rats.

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