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Research Article

Evaluation of hepatoprotective and antioxidant activity of *Sonerila tinnevelliensis* Fischer (Melastomataceae) whole plant - CCl_4 induced hepatotoxicity in rats

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ABSTRACT

CCl_4 intoxicated rats showed significant elevation in serum enzymes, bilurubin and lipid peroxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase activity. Treatment with ethanol extract of *Sonerila tinnevelliensis* whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100mg/kg) treated group. Thus the present study ascertains that the ethanol extract of *Sonerila tinnevelliensis* whole plant possesses significant hepatoprotective activity.

Key Words: *Sonerila tinnevelliensis,* Hepatoprotective activity, Silymarin, Bilirubin, CCl_4.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion so it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways for growth, fight against disease, nutrient supply energy provision and reproduction [1].

Liver disease is still a worldwide health problem; unfortunately, synthetic drugs used in the treatment of liver disease are in adequate and sometimes can have serious side effects [2]. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is much drug available for the treatment of liver disorders [3].

Liver diseases are mainly caused by toxic chemicals, (certain antibiotics, chemotherapeutics, peroxidised oil, aflotoxin, CCl_4, chlorinated hydrocarbons etc.) excess consumption of alcohol, infections and autoimmune disorder [4].

Sonerila tinnevelliensis Fischer. is used to cure liver diseases and gastrils. Its leaf extract is orally administered to cure body swelling by Kanikaran. Decoction of fresh leaves is consumed on an empty stomach once in a day to get relief from rheumatic complaints [5]. Hence the aim of the present study was to investigate the hepatoprotective activity of ethanol extract of *Sonerila tinnevelliensis* whole plant on CCl_4 induced liver toxicity in rats.

MATERIALS AND METHODS

Plant Material

The well grown and healthy whole plant of *Sonerila tinnevelliensis* Fischer were collected from natural forests of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extracts 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 ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures [6-8].The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

Animals

Normal healthy male Wistar albino rats (180-240g) 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 [9]. 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 50, 100 and 2000 mg/kg body weight.

Experimental Design

In the investigation, a total of 25 rats (CCl_4 hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

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

Group II: CCl_4 hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl_4 for 14 days.

Group III: Liver injured rats received ethanol extract of whole plant of *S. tinnevelliensis* at the dose of 200mg/kg body weight for 14 days.

Group IV: Liver injured rats received ethanol extract of whole plant of *S. tinnevelliensis* at the dose of 400mg/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 3000g for 10 minutes. Serum protein [10] and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), total, conjugated bilirubin, unconjugated bilirubin were determined as per the standard procedures [11,12]. Liver homogenates (10%W/V) were prepared in ice cold 10mM 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 Pal *et al* [13]. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD) were also assayed in liver homogenates as per the standard procedures [14,15].

Statistical Analysis

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

RESULTS

The effects of whole plant of *S. tinnevelliensis* extract on the body weight of the rays are shown in table 1. There was significant (p<0.01) decrease in body weight in CCl_4 intoxicated rats (Group II) compared with the normal control group (Group I).

The effect of ethanol extract of *S. tinnevelliensis* on serum total protein, albumin, globulin, A/G ratio, serum GOT, GPT and ALP in CCl_4 intoxicated rats are summarized in table 2.

There was a significant (p<0.01) increase in serum GOT, GPT and ALP levels in CCl_4 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 8.04 g/dl and 4.13 g/dl in CCl_4 intoxicated rats from the levels of 8.18 g/dl and 4.68 g/dl respectively in normal group. The ethanol extract of *S. tinnevelliensis* whole plant at the dose of 200 mg/kg and 400 mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

The effect of ethanol extract of *S. tinnevelliensis* on total, conjugated and unconjugated bilirubin is shown in table 3. A significant elevation of total, conjugated and unconjugated bilirubin in the serum of CCl_4 intoxicated group (Group II) when compared to normal control (Group I).The extract of *S. tinnevelliensis* at the dose 200 and 400 mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III).

The decrease in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin were found to be greater in standard silymarin (group IV) followed by Group (III) (Table 3).

The effects of ethanol extract of *S. tinnevelliensis* whole plant on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Glutathione reductase (GRD), Superoxidase (SOD) and Catalase activities are shown in table 4. LPO level was significantly (p<0.01) increased and GPx, GRD, SOD and CAT were significantly (p<0.01) decreased in CCl_4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *S. tinnevelliensis* at the dose of 200 and 400mg/kg significantly decreased the elevated LPO levels and restored GPV, GRD, SOD and CAT levels towards the normal levels in a dose dependent manner, The results are well comparable with silymarin (standard drug) treated group.

DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats, against CCl_4 as heptotoxin, to prove its claim in folklore practice against liver disorder[16]. CCl_4 is a widely used experimental hepatotoxicant, is biotransformed 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, disturb Ca^{2+} haemostasis and finally results in cell death [17]. Animals of Group II significantly loss their body weight as compared to normal control group (Group I). Animals of Group III and IV showed significantly increased in body weight as compared to Group II. These findings suggested the extract administrated has significantly neutralized the toxic effects of CCl_4 and helped in regeneration of hepatocytes [18].

Estimating the activities of serum marker enzymes like SGOT, SGPT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream[19]. In the present study, treatment with *S. tinnevelliensis* whole plant extract attenuated the increase in the activities of SGOT, GPT and ALP produced by CCl_4 indicating the *S. tinnevelliensis* whole plant extract protects liver injury induced by CCl_4 toward normalization, silymarin, a prototype hepatoprotective agent also showed similar changes.

Bilirubin is the main bile pigment that is formed by the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile [20]. Malfunctioning of the liver was evidenced by the significantly increase (p<0.01) in the level of unconjugated bilirubin in the serum of the group treated with only CCl_4 when compared to normal control group. Increase in the level of unconjugated bilirubin in the blood stream may result from a defect in the function of the liver to conjugate the bilirubin being produced. The significant reduction of unconjugated bilirubin level in the serum when CCl_4 was simultaneously administrated with the ethanol extract of *S. tinnevelliensis* when compared with the administration of CCl_4 alone indicates that conjugating function of the liver was improved. The reduction of the unconjugated bilirubin levels by the extracts may activate the constitutive androstance receptor (CAR) which is a key regulator in bilirubin clearance in the liver [21]. The primary function of CAR is the bilirubin clearance pathway is to direct coordinate response to the elevated levels of bilirubin by increasing the hepatic expressoin of each component of the pathway [22].

The simultaneous administration of CCl_4 with ethanol extract of *S. tinnevelliensis* was significantly reduced (p<0.01) the level of serum total protein when

compared with that of the CCl_4 treated group suggests the potential of the extract is clearing bilirubin from the serum when its level elevated. The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of ethanol extract of *S. tinnevelliensis* whole plant reversed these changes may be due to the increase in protein synthesis. This indicates the hepatoprotective activity of *S. tinnevelliensis* whole plant against damage by CCl_4. Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl_4 administration. In the present study, the elevation in the levels of end products of lipid peroxidation in the liver of rat treated with CCl_4 was observed. The increase in MDA (Malonialdehyde) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent the formation of excessive free radicals. Treatment with the extract of *S. tinnevelliensis* significantly reversed these changes.

SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage [23]. In the present study, it was observed that the extract of *S. tinnevelliensis* significantly (p<0.01) increased the hepatic SOD activity in CCl_4 induced liver damage in rats. Extract of *S. tinnevelliensis* can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant which decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [24]. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of extract of *S. tinnevelliensis* increased the activities of CAT in CCl_4 induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from CCl_4 intoxication. GRD plays a role in maintaining adequate amount of GSH. Accordingly, the GRD results in decreasing GSH in CCl_4 intoxicated rats, the activity of GRD is significantly (p<0.01) decreased. However, ethanol extract of *S. tinnevelliensis* with 200 and 400mg/kg body weight brought the activity of GRD towards normalization.

In conclusion, the results of this study revealed that the ethanol extract of *S. tinnevelliensis* whole plant has a potent hepatoprotective activity against CCl_4 induced hepatic damage in rats. The enhanced level of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanisms of whole plant of *S. tinnevelliensis* ethanol extract, it prevents the development of liver damage induced by CCl_4.

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Table 1
Effect of *Sonerila tinneveliensis* whole plant extract on the body weight of the rats before and after treatment in the normal, liver damaged and drug treated rats.

.Group	Dose	Initial Body weight (Gm)	Final Body weight (Gm)	Mean weight Gain(G↑)/ loss(L↓) (Gm)	% Difference
I	0.9% Saline	193.24±6.56	213.54±9.34	20.30↑	10.51
II	0.9% Saline	209.66±7.84	184.65±9.16**	25.01	11.93
III	200(mg/Kg)	213.54±9.64	208.51±8.54ns	5.03	2.36
IV	400(mg/Kg)	204.45±7.93	210.84±5.89a	6.39↑	3.13
V	100(mg/Kg)	207.11±9.34	214.83±9.16aa	7.72↑	3.73

Values are mean ± SEM of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. p<0.05; **p<0.01; as compared with normal Control to liver damaged control: a P<0.05; aa P<0.01 as compared with liver damaged control to drug treated animal; ns: not significant

Table 2
Effect of *Sonerila tinneveliensis* whole plant extract 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	Parameters						
	T.Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	8.18±1.21	4.68±1.05	3.50±0.14	1.3:1	19.36±1.27	21.43±1.92	193.46±3.81
II	8.04±1.62	4.13±0.92	3.91±0.51	1.1:1	116.54±4.82***	124.16±5.43***	294.62±4.88***
III	8.24±1.92	4.84±0.84	3.40±0.44	1.4:1	43.66±3.82*aa	34.98±4.86aa	215.16±6.44aa
IV	8.31±1.34	4.92±1.24	3.39±0.23	1.5:1	31.84±1.33ns aa	26.84±3.21aaa	198.65±4.88aaa
V	8.24±1.95	4.88±1.24	3.36±0.22	1.5:1	22.94±1.86aaa	20.65±1.94aaa	173.81±4.54aaa

Values are mean ± SEM of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; **p<0.01; as compared with normal Control to liver damaged control: a p<0.05; aa p<0.01 aaa p<0.001 as compared with liver damaged control to drug treated animal. ns: not significant.

Table 3.
Effect of *Sonerila tinneveliensis* whole plant extract on the serum Total, conjugated and unconjugated bilirubin levels in the normal control, liver injured and drug treated rats.

Groups	Parameters			
	Total Bilirubin (µmol/L)	Conjugated (µmol/L)	Unconjugated (µmol/L)	GGTP (U/L)
I	0.83±0.07	0.21±0.05	0.62±0.03	7.93±0.54
II	4.15±0.81***	3.06±0.72***	1.09±0.12*	26.84±1.26**
III	1.93±0.54a	1.24±0.26*a	0.69±0.04ns	13.42±1.04a
IV	1.08±0.36aa	0.82±0.12aa	0.26±0.01aa	9.27±0.86aa
V	0.96±0.04aaa	0.76±0.08aa	0.20±0.03aa	6.94±0.24aa

Values are mean ± SEM of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; **p<0.01; as compared with normal Control to liver damaged control: a p<0.05; aa p<0.01 as compared with liver damaged control to drug treated animal.
ns: not significant

Table 4
Effect of *Sonerila tinneveliensis* whole plant extract on serum LPO, GPX, GRD, SOD, CAT and GSH activity in the normal control, liver injured and drug treated rats.

Groups	Parameters					
	LPO (n mole of MDA/mg protien)	GPX (u/mg Protein)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)	GSH (u/mg)
I	2.091±0.014	3.948±0.112	0.504±0.014	0.294±0.014	4.062±0.018	36.95±0.14
II	6.816±0.094**	1.946±0.106**	0.241±0.027**	0.112±0.016**	2.091±0.016**	11.94±0.09**
III	3.164±0.069ns	2.684±0.119*	0.403±0.016*a	0.198±0.024* a	3.124±0.081a	24.81±0.18*
IV	2.241±0.046a	4.016±0.241aa	0.495±0.024aa	0.281±0.017aa	3.986±0.054a	32.16±0.21aa
V	2.148±0.071aa	4.121±0.415aa	0.498±0.016aa	0.281±0.016aa	4.112±0.076aa	29.68±0.16aa

Values are mean ± SEM of 6 animals in each group.Statistical analysis ANOVA followed by Dunnett t-test. *p<0.05; **p<0.01; as compared with normal Control to liver damaged control: a p<0.05; aa p<0.01 as compared with liver damaged control to drug treated animal.
NS: not significant

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