Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 5, Issue 1, 2013

Research Article

EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *MELASTOMA MELABATHRICUM* L. LEAF- CCL_4 INDUCED HEPATOTOXICITY IN RATS

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Received: 17 November 2012, Revised and Accepted: 21 December 2012

ABSTRACT

The effect of ethanol extract of *Melastoma melabathricum* leaf was evaluated in carbon tetrachloride induced hepatotoxicity in rats. Liver necrosis was produced by administrating single dose of carbon tetrachloride (CCl_4 2.5ml/kg body weight with normal saline) for 14 days. The liver damage was evidenced by elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), total, conjugated, unconjugated bilirubin, gamma glutamyl transferase (γGTP) and liver lipid peroxidation (MAD) and reduced the liver antioxidant such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD). Ethanol extract of *Melastoma melabathricum* pretreatment (250 and 500 mg/kg body weight) significantly ($p<0.01$; $p< 0.05$) reduced CCl_4 induced elevation of SGOT, SGPT, ALP, total, conjugated, unconjugated bilirubin, γGTP and LPO (MAD). While the reduced concentration of SOD, CAT, GPx and GRD were reversed. Silymarin (100 mg/kg body weight) a known hepatoprotective drug showed similar results.

Keywords: *Melastoma melabathricum*, Hepatoprotective, ALP, Bilirubin, MAD.

INTRODUCTION

Liver is a versatile organ and plays a vital role in the metabolism of chemicals such as dietary uptake, toxins in body and such other substances in the circulatory system. The environmental pollutions with chemicals and other hazardous materials continuously expose the liver to a variety of xenobiotic agents. In spite of a great studies made by modern system of medicine, there are hardly few curative or therapeutic agents for liver affections which may stimulate the liver functions[1]. There is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM). Some phytoconstituents have significant hepatoprotective activity. Many traditional remedies employ herbal drugs for treatment of liver ailments[2-5].

Melastoma melabathricum belongs to the Melastomataceae family. It is also called the Singapore Rhododendron or Sendudok. It is a erect shrub or small tree 1.5 to 5m tall. It was traditionally used to treat diarrhoea, dysentery, leucorrhoea, hemorrhoids, wounds, and infection during confinement, toothache, flatulence, sore legs, and thrush and also it is used by the Jah hut people in Malaysia to cure diarrhoea[6]. However, inspite of traditional use, pharmacology of its leaves has not yet been explored scientifically. Literature reviews indicated that the hepatoprotective activity of leaves of *Melastoma melabathricum* has not been scientifically evaluated so far. An active and safe drug is needed for the treatment of jaundice. In view of this, the present study was aimed at evaluating the hepatoprotective activity of leaves of *Melastoma melabathricum* against CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material

The leaves of *Melastoma melabathricum* L. were collected from Daudeli,Joide Taluk, Hubli District, North Karnataka. With the help of local flora, a voucher specimen (VOCB 1637) was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

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 [7-9]. 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 [10]. 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 *Melastomma melabathricum*at the dose of 250mg/kg body weight for 14 days.

Group IV: Liver injured rats received ethanol extract of whole plant of *Melastomma melabathricum*at the dose of 500mg/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 [11] 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) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel[12]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [13].

Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw [14]. The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gamma-glutamyltransferase (GGT) was estimated by the method of Szasz[15]. 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 Okhawa[16]. Enzymatic antioxidants, superoxide dismutase (SOD)[17]catalase (CAT)[18,19] and non enzymatic antioxidant glutathione peroxidase (GPx)[20] and glutathione reductase (GRD) [21] 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. $p<0.05$and $p<0.01$ were considered as statistical significance using SPSS Software.

RESULTS

The ethanol extract of leaves of *Melastomma melabathricum* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoids, saponins, steroids, phenols, tannins and xanthoproteins. The ethanol extract did not show any sign and symptoms of toxicity and mortality upto 2000mg/kg dose. Table 1 shows the body weight of the normal, liver damaged and drug treated rats. The effect of ethanol extract of *Melastomma melabathricum* on serum total protein, albumin, globulin, A/G ratio, serum transaminases, alkaline phosphatases 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 6.78g/dl and 4.37g/dl in CCl_4 intoxicated rats from the levels of 8.14g/dl and 4.50g/dl respectively in normal group. Ethanol extract of *Melastomma melabathricum* at the dose of 250 and 500mg/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 *Melastomma melabathricum* on total, conjugated, unconjugated bilirubin and gamma-glutamyltransferase is shown in Table 3. A significant elevation of total, conjugated, unconjugated bilirubin and gamma-glutamyltransferase in the serum of CCl_4 intoxicated group (Group II) when compared to normal control (Group I). The ethanol extract of *Melastomma melabathricum* at the dose 250 and 500mg/kg reduced the levels of total, conjugated, unconjugated bilirubin and gamma-glutamyltransferase (Group III and Group IV). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and gamma-glutamyltransferase were found to be greater in standard silymarin (Group V) followed by Group IV and Group III (Table 3).

Table 1: Effect of *Melastoma melabathricum* Leaf extract on the body weight of the rats before and after treatment in the normal, liver damaged and drug treated rats.

Groups	Dose	Initial Body weight (Gm)	Final Body weight (Gm)	Mean weight Gain (G↑) / loss(L↓) (Gm)	% Difference
I	0.9% Saline	198.45±6.34	211.65±6.31	13.2↑	6.65
II	0.9% Saline	208.14±9.37	189.21±4.37	18.93↓**	9.09
III+IV	100(mg/kg)	198.33±7.35	208.38±5.67	10.05↑^{a}	5.06
	200(mg/kg)	203.16±8.18	212.97±10.13	9.81↑^{aa}	4.82
V	100(mg/kg)	201.44±8.15	214.98±7.08	13.54↑^{aa}	6.72

Values are mean ± SD of 5 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

Table 2: Effect of *Melastoma melabathricum* Leaf extract on the protein, albumin, globulin concentration and enzyme activity of serum GOT, GPT, and ALP in the normal , liver damaged and drug treated rats.

Groups	Parameters						
	T.Protein (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	8.14±0.16	4.56±0.12	3.58±0.11	1.27:1	11.26±0.31	13.92±0.86	134.61±4.26
II	6.78±0.15*	4.37±0.21	2.41±0.09*	1.56:1	58.91±2.56*	54.86±1.93**	211.63±7.36*
III	7.98±0.26	4.15±0.33	3.83±0.24	1.08:1	19.84±1.15^{a}	16.05±1.13^{a}	149.66±6.54^{a}
IV	8.59±0.11	4.93±0.26	3.66±0.18	1.28:1	13.29±1.88^{aa}	10.27±0.89^{aa}	129.55±4.86^{aa}
V	8.07±0.17	4.55±0.18	3.51±0.74	1.29:1	12.88±0.98^{aa}	10.23±0.85^{a}	142.81±4.16^{a}

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p<0.01$ Compared normal control vs liver injured rats a: $p < 0.05$; aa $p<0.01$ Compared liver injured rats vs drug treated

Table 3: Effect of *Melastoma melabathricum* Leaf extracts on the serum Total, conjugated and unconjugated bilirubin and GGT levels in the normal control, liver injured and drug treated rats.

Groups	Parameters			
	Total Bilirubin (µmol/L)	Conjugated (µmol/L)	Unconjugated (µmol/L)	GGT (U/L)
I	0.61±0.04	0.21±0.06	0.40±0.04	10.93±0.21
II	3.05±0.31**	1.29±0.07**	1.76±0.14**	26.23±0.72**
III	0.98±0.06^{a}	0.24±0.01^{a}	0.74±0.01ns	14.09±0.73^{a}
IV	0.74±0.05^{aa}	0.21±0.04^{aa}	0.53±0.02^{a}	8.27±0.54^{aa}
V	0.76±0.06^{a}	0.24±0.07^{aa}	0.62±0.05^{a}	8.93±0.13^{a}

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p<0.01$ Compared normal control vs liver injured rats a: $p < 0.05$; aa $p<0.01$ Compared liver injured rats vs drug treated ns-Not significant.

Table 4: Effect of *Melastoma melabathricum* Leaf extract on serum LPO, GPx, GRD, SOD and CAT 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)
I	2.051±0.054	0.324±0.005	3.129±0.026	3.922±0.059	0.486±0.051
II	4.892±0.073**	0.109±0.011**	1.101±0.075*	1.121±0.084*	0.098±0.012**
III	2.544±0.061[a]	0.291±0.036[a]	2.883±0.051[a]	3.306±0.074[a]	0.384±0.073[a]
IV	2.113±0.089[aa]	0.384±0.016[aa]	3.514±0.039[aa]	3.809±0.024[aa]	0.471±0.053[a]
V	2.098±0.012	0.312±0.034[a]	2.993±0.018[a]	4.894±0.026[aa]	0.493±0.019[aa]

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p<0.01$ Compared normal control vs liver injured rats a: $p < 0.05$; aa $p<0.01$ Compared liver injured rats vs drug treated.

The effects of ethanol extract of *Melastomma melabathricum* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Glutathione reductase (GRD), Superoxide dismutase (SOD) and Catalase (CAT) activity is shown in Table 4. 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 CCl_4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *Melastomma melabathricum* at the doses of 250 and 500 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels towards the normal levels in a dose dependent manner. The results are well comparable with silymarin (standard drug) treated group.

DISCUSSION

It is well established that CCl_4 induces hepatotoxicity by metabolic activation, therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome P_{450} system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl_3). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl free radicals lead to elicit lipid peroxidation, the destruction of Ca^{2+}homeostasis and finally, results in cell death [22,23]. These results in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme, metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphate activation, leading to liver damage[24-28]. Hepatotoxic compounds like CCl_4 are known to cause marked elevation in serum enzyme activities. In the present study, treatment with *Melastomma melabathricum* leaf extract attenuated the increase in the activities of SGOT, SGPT and ALP produced by CCl_4 indicating that *Melastomma melabathricum* leaf extract protects liver injury induced by CCl_4 towards normalization. Silymarin, a prototype hepatoprotective agent also showed similar charges.

Bilirubin is the main bile pigment that is form 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[29]. Malfunctioning of the liver was evidenced by the significant 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. Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced[30]. The significant reduction ($p< 0.05$) of unconjugated bilirubin level in the serum when CCl_4 was simultaneously administrated with the ethanol extract of *M. melabathricum* when compared with the administration of CCl_4 alone indicates that the conjugating function of the liver was improved. The reduction of the unconjugated bilirubin level by the ethanol extract suggest that the extracts may activate the constitutive androstance receptor (CAR) which is a key regulator in bilirubin clearance in the liver[31]. The primary function of CAR is the bilirubin clearance pathway is to direct cooridnate response to elevated levels of bilirubin by increasing the hepatic expressive of each component of the pathway[31].

The ability of simultaneous administration of CCl_4 with ethanol extract of *M. melabathricum* to significantly reduce ($p< 0.01$) the level of serum total bilirubin 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[32].

Since the results obtained for the serum total protein and albumin concentrations followed the same trend, it thus implicated the same mechanism by which the ethanol extract of *M. melabathricum* exerts its effect on these parameters. The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of ethanol extract of *M. melabathricum* leaf reversed these changes may be by increasing protein synthesis. This indicates the hepatoprotective activity of *M. melabathricum* leaves against damage by CCl_4. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells[33].

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 are parallel to those of amino transferases. The acute damage caused by CCl_4 increased the γ-glutamyl transferase level but the same attains the normal after *Melastoma melabathricum* treatment due to its antioxidant activity.

Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl_4 administration. In the present study the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with CCl_4 were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with ethanol extract of *M. melabathricum* significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection by *M. melabathricum* extract is due to its antioxidant effect.

The enzyme antioxidant defense system is the nature protector against lipid peroxidation. 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[34]. In the present study, it was observed that the ethanol extract of *M. melabathricum* significantly ($p< 0.01$) increased the hepatic SOD activity in CCl_4 induced liver damage in rats. This show ethanol extract of *M. melabathricum* can reduce reactive free radicals that might lesson oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposed hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals[35]. 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 ethanol extract of *M. melabathricum* 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 in toxication.

To prevent lipid peroxidation, it is very important to maintain the level of GSH, GSSG is reduced to GSH by GR, which is NADPH-dependent. It plays a role in maintaining adequate amounts of GSH. Accordingly, the reduction of GR results in decreasing GSH[36]. In CCl_4 intoxicated rats, the activity of GR is significantly ($p<0.05$) decreased. However, ethanol extract of *M. melabathricum* with 250 and 500 mg/kg bodyweight brought the activity of GR towards of normalization.

In conclusion the present study has demonstrated that ethanol extract of *M. melabathricum* has hepatoprotective effect against CCl_4 induced hepatotoxicity in rats. It is suggested that, saponins in *M. melabathricum* leaf play on important role an antioxidant for prevention of oxidative hepatic damage. Furthermore, the flavonoids and saponins of *M. melabathricum* may able to stabilize reactive oxygen species by reacting with them and oxidizes subsequently to more stable and less reactive radicals. The enhanced levels of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanism of *Melastoma melabathricum* ethanol extract in prevents the development of liver damage induced by CCl_4.

ACKNOWLEDGEMENT

Thanks to Dr. Sampathraj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur for their assistance in animal studies.

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