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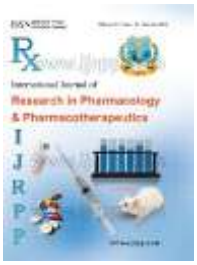

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Evaluation of hepatoprotective and antioxidant activity of *Merremia turpethum*

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the methonalic extract of Merremiaturpethum(MEMT) in Wistar albino rats. Protective action of MEEC was evaluated using animal model of hepatotoxicity induced by acetaminophen (2 g/kg). Liver maker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Histopathology was also studied. Levels of aspartate aminotransferase (AST), alanine aminotransferace (ALT), alkaline phosphatase (ALP) and total bilirubin were increased and the levels of total protein were decreased in acetaminophen treated rats. The potency of methanol extract of the plant showed rich antioxidant properties.From this study it can be concluded that the (MEMT) showed significant hepatoprotective and antioxidant action.

Keywords: Antioxidant, Anthraquinone, Saponin, alkaloids, flavonoids, tannins,saponins, *Merremia turpethum*, DPPH, hydrogen peroxide radical scavenging activity.

INTRODUCTION

Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radicals. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism. Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA has been implicated in the pathogenicity of major diseases like cancer, rheumatoid arthritis etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by free radicals by interfering with the oxidation process through radicals scavenging and chelating metals ions.

Acetaminophen (paracetamol), a most commonly used analgesics, it effectively reduces fever and mild-to moderate pain, is considered to be safe at therapeutic doses. However, acetaminophen

overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals. Most of the experiments aimed to elucidate the mechanism of acetaminophen toxicity were performed on animal model both in vivo and in vitro. When taken at supratherapeutic doses, acetaminophen causes centrilobular hepatocyte degeneration and necrosis in rodents and humans. In response to injury with Paracetamol and other centrilobularhepatotoxicants, there is a recovery phase in which hepatocytes are stimulated to repopulate the liver lobule. Resistance to a different toxicant (heteroprotection) has also been observed.

The mechanism(S) underlying the resilience of proliferating hepatocytes to further toxicity is not completely known. A small amount of acetaminophen is metabolized together with cytochrome P450. As a result, N-acetyl-p-benzoquinoneimine (NAPQI) appears in the body system. Both these compounds are very active chemically and their chemical structures indicate that they are capable of taking part in free radicals reactions. Consequently, acetaminophen overdose can lead to a number of unfavorable consequences, especially those affecting the liver. A large dose of this drug causes depletion of the cellular glutathione (GSH) level in liver because NAPQI reacts rapidly with glutathione, which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction. Thus, the GSH depletion, especially occurring in acute hepatotoxicity, affects liver or death. Since oxidative stress and GSH depletion contributed paracetamol induced liver injury; the agent(s) with antioxidant property and/or GSH reserving ability may provide preventive effect against the progression of lipid peroxidation and hepatocellular injury.

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 effect of synthetic agent, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatroprotective activity.

Plant derived natural products such as flavonoids, terpenoids, amino acids and Vitamin C etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotectiveactivity. These have 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. Antioxidant plays an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degeneration diseases. Realizing the fact, this research was carried out to evaluate the antioxidant and hepatoprotective activity of *Merremia turpethum* extract against acetaminophen – induced hepatic damage in rats.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Ethno botanical survey on medicinal plants found in the Ananthapuramu district was extensively studied and two folklore medicinal plants *Merremia turpethum* were identified for the present investigation[3]. Based upon the information collected on the medicinal plant were collected, identified and authentication was made from the local botanist.

Drugs and chemicals

Acetaminophen (APAP), Pyridine (C_5H_5N), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), dihydrogen potassium phosphate anhydrous (KH_2PO_4) and thiobarabituric acid (TBA)were purchased from Merck India Ltd (Mumbai, India), Diagnostic kits for the serum aspartate aminotrasferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum bilirubin, bovine serum albumin (BSA), trichloro acetic acid (TCA), thiobarbituric acid-reacting substances (TABRS), reduced glutathione (GSH), sodium pyrophosphate, ethylentdiamine tetra acetic acid disodium salt (EDTA) 5,5 – dithiobis (2-

nitrobenzoic acid) (DTNB), b-nicotinamide adenine dinucleotide hydrogen (NADH) were obtained from Sigma Chemical (St. Louis, MO, USA). All other chemicals and reagent were of highest purity commercially available.

Animals

Experimental animals Wister albino rats weighing 175-250 g used in the present studies. The animals were housed in plypropylene cages with sterile inert husk materials as bedding. All the animals were kept under standard environment condition at $23+2^0$C (12 h light / 12 h dark cycle at room temperature) and maintained on commercial pellet diet, it was supplied by, "HINDUSTAN LEVER" Limited Mumbai, marked under the trade name "Gold mohar" feeds, water was provided ad libitum. The rats were kept in animal house for ten days before starting the experiments.

Collection and preparation of plant materials

The aerial parts of the plant *Merremia turpethum*., belongs to the family Euphorbiaceaewas collected. The plant **was** collected from Kolli Hills at Namakkal District, Tamilnadu, India. The aerial parts of the plants were collected and dried in shade and powdered to obtain coarse powder. The coarse powder material (500 g) was extracted with methanol (95% v/v) by using soxhlet apparatus. The methanol extract was concentrated in vacuo and kept in a vacuum desiccator for complete removal of solvent. The yield was 11.35% w/w with respect to dried powder. The extract was subjected to preliminary qualitative tests to identify the various phytoconstituents present. It was observed that methanolic extract contained carbohydrates, terpenoids, tannins, flavonoids, steroids and glycosides.

Experimental design

Acetaminophen (APAP) obtained from sigma Aldrich, India. The animals were divided into four groups consisting of six animals each for different experiments. Group I rats served as normal control, Group II (intoxicated group) received orally with a single dose of acetaminophen (2 g/kg, bw,p.o,) diluted with sucrose solution (40% w/v). Group III rats were pre-treated with Silymarin commercial drug (100 mg/kg, bw, p.o,) for 10 days, followed by rats intoxicated with acetaminophen. Group IV were pre-treated with the MEEC (400 mg/kg, bw, p.o) for 10 days, followed by rats were intoxicated with acetaminophen. The animals were anesthetized 24 h after the administration of acetaminophen using ether anesthesia. Blood was then drawn by cardiac puncture to determine the serum ALT, AST, ALP, bilirubin activities; finally the animals were then sacrified. Liver was dissected out for the determination of antioxidant (LPO, SOD, GPX, CAT and GSH) status. The liver was then subjected to histopathological examination.

Serum biochemical assays

At the end of the experiment, the blood was collected by cardiac puncture from the ether anesthetized rats. The blood sample was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30^0C for 15 min and utilized for the estimation of various biochemical parameters namely ALT, AST, ALP, total bilirubin and total protein.

Determination of antioxidant activity in liver

After collection of blood samples the rats in different groups were sacrified and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15M Tris-Hcl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15M Tris-Hcl buffer and processed for the estimation of lipid peroxidation. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40^0C. The supernant thus obtained was used for estimation of SOD, CAT and GPX activities.

Histopathological examination

Liver pieces were preserved in 10 % formaldehyde solution. The pieces of liver processed and

embedded in paraffin wax. Sections of about 4-6 microns were made and stained with hematoxylin and eosin and photographed.

Statistical analysis

The results were expressed as mean ± SEM and were analyzed for statistically significant difference using one-way ANOVA followed by Bonferroni's multiple comparsion tests (BMCT) post hock test. The data were analyzed with SPSS version 16 software (SPSS Inc., Chicago, USA). The difference showing a level of $p<0.05$ was considered to be statistically significant.

EXTRACTION PROCESS FOR THE SELECTED PLANT MATERIALS

The selected plant material *Merremia turpethum* were subjected for the hot non-sequential extraction process by using soxhlet apparatus [7]. The plant material was extracted using methanol as a solvent.

PREPARATION OF EXTRACTS

The plant powders were subjected to methanolic solvent extrac. These extracts were further concentrated by using Buchi (R-200) Rotavapour. The concentrated semi-solid extracts were stored in air tight screw cap vials and kept in refrigerator till further use.

RESULTS & DISCUSSIONS

DPPH Free Radical Scavenging Activity

The plant extract was dissolved in small quantity of water to get a concentration of (1mg/mg), 2.5ml this solution was added with 1ml (0.1mM) DPPH (in methanol) solution to get a serial dilution in the concentration ranging from (25 to 300µg/ml) with methanol[8] as shown in Table.1. The resulting solution as shown in fig.1 was incubated in the room temperature for 20 minute and the all the solution was measured at 517nm spectrophotometricaly against the reagent blank. Ascorbic acid was used as the reference compound [9]. The IC50 and percentage inhibition was calculated by using the following equation.

$$\% I \text{ (percentage inhibition)} = [(Ac - At)/ At] \times 100$$

Where,

Ac = was the absorbance of the negative control (Blank, without extract or standard),

At -was the absorbance of all the extract and reference compound.

Table.1: DPPH radical scavenging activity of *Merremia turpethum*

Extract / Standard	Conc. (µg/ml)	Absorbance (MEAN ± SD) n=3	% Inhibition (MEAN ± SD) n=3	IC_{50} (µg/ml)
Control	- -	0.027	- -	--
Ascorbic Acid	25	0.978 ± 0.002	10.056 ± 0.112	12.933
	50	0.877 ± 0.003	20.730 ± 0.195	
	100	0.941 ± 0.005	38.371 ± 0.297	
	150	0.976 ± 0.004	42.004 ± 0.213	
	200	0.853 ± 0.002	58.951 ± 0.086	
	300	0.856 ± 0.011	64.382 ± 0.618	

Methanolic extracts of *Merremia turpethum*	25 50 100 150 200 300	0.919 ± 0.040 0.897 ± 0.004 0.810 ± 0.005 0.872 ± 0.005 0.940 ± 0.006 0.931 ± 0.005	25.562 ± 2.241 29.607 ± 0.203 33.352 ± 0.265 36.629 ± 0.297 44.064 ± 0.309 50.169 ± 0.297	16.507

HYDROGEN PEROXIDE ANTIOXIDANT ACTIVITY

40mM of hydrogen peroxide solution was preparedusing phosphate buffer solution (pH 7.4). Extract was dissolved in small quantity of distilled water to get a concentration of (100 µg/ml). This solution was added to 0.5ml of 40mM solution of hydrogen peroxide to get a concentration ranging from (25 - 300µg/ml) was made adjusted using distilled water as shown in Table.2. This resulting solution as shown in fig.2 was measured at 230nm and the percentage inhibition was determined by using the above mention formula, Gallic acid was used as reference standard.

Table.2: Hydrogen peroxide scavenging activity of *Merremia turpethum*

Extract / Standard	**Conc. (µg/ml)**	**Absorbance (MEAN ± SD) n=3**	**% Inhibition (MEAN ± SD) n=3**	**IC $_{50}$ (µg/ml)**
Control	- -	0.027	- -	--
Gallic Acid	25 50 100 150 200 300	0.542 ± 0.006 0.605 ± 0.004 0.872 ± 0.004 0.931 ± 0.006 0.968 ± 0.001 0.905 ± 0.007	9.975 ± 1.728 18.100 ±1.086 36.792 ± 1.018 47.814 ±1.728 54.839 ±0.269 61.774 ±1.882	107.087
Etyhanolic extract of *Merremia turpethum*	25 50 100 150 200 300	0.750 ± 0.006 0.807 ± 0.004 0.873 ± 0.005 0.838 ± 0.004 0.975 ± 0.005 0.996 ± 0.006	5.914 ± 1.497 10.473 ± 1.172 21.523 ± 1.353 32.111 ± 1.086 42.957 ± 1.232 54.104 ± 1.529	97.424

Evaluation of Hepatoprotective activity

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the MEEC showed moderate presence of flavanoids and very highly present of saponins, phytosterols, phenolic compound, tannis.

Serum biochemical parameters

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phophatase (ALP), total bilirubin were significantly ($p<0.05$) increased and the levels of total protein were significantly ($p<0.001$) decreased in acetaminophen treated rats when compared to control group (Table 1). Administering silymarin and MEEC (200 mg/kg, bw, p.o, and 400 mg/kg, bw, p.o, respectively) reduced the elevated levels of AST, ALT, ALP and total bilirubin levels as well as restore the levels of total protein towards normalcy when compared to acetaminophen treated rat.

Hepatic oxidative stress parameters

Lipid peroxidase (LPO) level was significantly ($p<0.001$) increased and the levels of GSH, CAT and SOD were significantly ($P<0.001$) decreased in acetaminophen treated rats when compared to control group. Administering silymarin and PGJ leaves (100 mg/kg bw, p.o, and 500 mg/kg bw, p.o, respectively) significantly ($p<0.05$) decreased the elevated levels of Lipid peroxidase (LPO) content as well as increased significantly ($p<0.001$) the antioxidant levels (Table 2).

Histopathological examination

Liver sections from control rats showed normal lobular architecture and normal hepatic cells with a well preserved cytoplasm, nucleus and nucleoli were defined. Whereas rats treated with acetaminophen showed marked regenerative activity in the form of binucleation, prominent nucleoli, nuclear, and kupffer cells were hyperplastic, no significant morphological changes were noted in liver of animals given only silymarin, as compared to that of animals in the group.

Table 1: Effect of methanol extract of *Merremia turpethum*on biochemical parameters in acetaminophen induced hepatic injury in rats.

S.No	Groups	AST(IU/I)	ALT(IU/I)	ALP(IU/I)	Total bilirubin (mg/dl)	Total protein (gm/dl)
I	Control	75.34±1.32	57.42±2.75	62.04±2.62	1.05±0.12	7.12±0.32
II	Acetaminophen (2 g/kg, bw, p.o)	107.83±2.31	83.18±2.82	103.22±4.12	2.52±0.82	3.84±0.41
IV	Acetaminophen (2 g/kg, bw, p.o) + MEMT (400 mg/kg, bw, p.o)	87.32±2.51**	59.32±2.15**	76.20±3.20**	1.92±0.04**	5.53±0.26**

Each value represents the mean ± SE of six animals, Significant difference at *P < 0.05 and **P < 0.001 compared with the acetaminophen treated group.

Table 2: Effect of methanol extract of *Merremia turpethum*on antioxidant activity in acetaminophen induced hepatic injury in rats.

S.No	Groups	LPO (nmol/mg protein)	GSH (µmol/mg protein)	SOD (µmol/mg protein)	CAT (µmol/mg protein)	GPX (µmol/mg protein
I	Normal control	1.85±0.04	23.83±2.02	63.52±2.04	24.06±2.08	18.90±1.26
II	Acetaminophen (2 g/kg, bw, p.o)	5.70±0.25	15.40±1.24	47.09±1.24	14.30±1.04	13.25±1.05

III	Acetaminophen (2g/kg,bw, p.o) + Silymarin (200mg/kg, bw, p.o)	2.02±0.04*	22.25±1.02*	62.30±2.31*	22.15±1.74*	17.32±1.18*
IV	Acetaminophen (2 g/kg, bw, p.o) + MEEC (400 mg/kg, bw, p.o)	1.82±0.04**	23.42±1.58**	63.28±1.69**	24.42±1.85**	18.32±1.82**

Each value represents the mean ± SE of six animals, Significant difference at *P < 0.05 and **P < 0.001 compared with the acetaminophen treated group.

CONCLUSION

It can be concluded that the antioxidant activity measured and therefore it is recommended to use for better assessment of the antioxidant activity of natural products. Several medicinal plants tested are rich sources of flavonoid compounds and free radical scavengers. Some medicinal plants thus can be considered as promising sources of natural antioxidants for medicinal and commercial uses.The results of hepatoprotectivestudy demonstrate that *Merremia turpethum* has a potent hepatoprotective action upon acetaminophen – induced oxidative stress and liver toxicity in rat. The hepatoprotective effect of *Merremia turpethum*can be correlated directly with its ability to reduce status. The finding of this study suggest that *Merremia turpethum* can be used as safe, cheap, and effective alternative protective agent in the management of liver diseases.

REFERENCES

[1] Preussmann, R. Hepatocarcinogens as potential risk for human liver cancer. In: Remmer, H., Bolt, H.M., Bannasch, P. Primary liver tumors. Lancaster: MTP Press; 1978; P. 11-29.

[2] Chattopadhyay, R.R. Possible mechanism of Hepatoprotective activity of Azadirachtaindica leaf extract . J.Ethnopharmacol 2003; 81, 217-219.

[3] Knight, T.R., Ho, Y S., Farhood, A., Jaeschke, H.Peroynitrite is a critical mediator of acetaminophen hepatotoxicity in murine livers; protection by glutathione. Journal of pharmacology and experimental Thereutics 2002; 303, 468-475.

[4] Yim, H.K., Jung, Y.S., Kim, S.Y., Kim, Y.C. Contrasting change in phase I and phase II metabolism of acetaminophen in mice pretreated with carbon tetrachloride. Basic and Clinical Pharmacology and Toxicology. 2006; (98), 225-230.

[5] Oz,HS., McClain, CJ.,Nagasawa, HT., Ray, MB., de Villiers, WJ., Chen, TS Diverse antioxidants protect against acetaminophen hepatotoxicity. Journal of Biochemical and Molecular Toxicology 2004; (18) 361-368.

[6] Roy, CK., Kamath, JV., Azad, M. Hepatoprotective activity of PsidiumguajavaL.leaf extract, Indian J Exp Bio2006; 44(4),305-311.

[7] Boaz M, Smetana S, Einstein WT et.al. Secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE): randomized placebo-controlled trial. Lancet 2000; 356: 1213-1218.

[8] Ahmad B, Azam S, Bashir S, Hussain F, chaudhary MI. Insecticidal, brine shrimp cytotoxicity, antifungal and nitric oxide free radical scavenging activities of the aerial parts of Myrsineafricana L. African Journal of Biotechnology 2011; 10(8):1448-1453.

[9] Sanchez-Moreno, C., Larrauri, J. and Saura-Calixto, F.,(1999) Free radical scavenging capacity of selected red and white wine, J. Sci.Food. Agric., 79, 1301-1304.

[10] Sian B A (2003). Dietary antioxidants—past, present and future? Trends in Food Sci. Technol. 14: 93-98.

[11] Shyur, L.F., Tsung, J.H., Chen, J.H., Chiu, C.Y. and Lo, P.C,(2005). Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. In. J App Sci& Engineering, 3, 195-2002.

[12] Sofowora, A. Medicinal plants and Traditional medicines in Africa. Spectrum books, Ibadan,1993. pp150.

[13] Sivastava SK, Srivastava SD, Chouksey BK. New constituents of Terminaliaalata. Fitoterapia

[14] 1999;70:390–394.

[15] Krishnaiah D, Sarbatly R, Nithyanandam RR. A review of the antioxidant potential of medicinal plant species. Food Bio prod Process 2011; 89:217–233.

[16] Pegg RB, Amarowicz N, Naczk M, Antioxidant activity of polyphenolics from a bearberry-leaf (Arctostaphylosuva-ursi L. Sprengel) extract in meat systems. ACS Symp Series909 2005.