Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (3):243-248
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Effects of *Polyalthiya longifolia* fruits extract on lipid profile and antioxidant status during DEN/PB induced hepatocellular carcinoma in rats

Jayaraman R.[1]* and A. J. M. Christina[2]

[1]*Karpagam University, Coimbatore, Tamil Nadu, India*
[2]*Taylors University, Malaysia*

ABTRACT

This study was designed to investigate the effects of methanolic extract of Polyalthiya longifolia fruits on N-nitrosodiethylamine/ Phenobarbital (DEN/PB) induced Hepatocellular damage with special reference to lipid profile and antioxidant status.Rats received with DEN/PB showed the elevated levels of total cholesterol ($p<0.05$), triglycerides (TG, $p<0.01$), free fatty acids (FFA, $p<0.05$), low density lipoprotein (LDL, $p<0.05$), very low density lipoprotein (VLDL, $p<0.05$) and decreased level of high density lipoprotein (HDL), urea and creatinine and glucose levels. Administration of MEPL 200,400 mg/kg.p.o to hepatocellular damage bearing rats orally for 28 days significantly altered the biochemical variations induced by DEN/PB in a dose dependent manner which indicates it's protective and lipid lowering effects during DEN/PB induced hepatocellular damage in rats. Moreover results from antioxidant studies revealed that, maximum percentage inhibition of DPPH radicals by MEPL was about 76% at 800 µg/mL whereas in the nitric oxide radical scavenging model, the maximum percentage inhibition is about 59% at 800µg/mL and in reducing power method, MEPL demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. Thus, it clearly indicates that MEPL might be beneficial in attenuating the elevated biochemical parameters during DEN/PB induced hepatocellular damage and the results suggested the ability of the extract to combat oxidative stress by quenching free radicals which reveals that, the attenuation due to its anti-oxidant property.

Keywords: *Polyalthiya longifolia*, N-nitrosodiethylamine, Phenobarbital, Hepatocellular damage, Antioxidants.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common life threatening malignancy which is mainly due to the damage of hepatocytes when they are exposure to hepatotoxins like N-nitrosodiethylamine and leads to mortality all around the world and requires immediate attention for the treatment (1). N-nitrosodiethylamine is a powerful hepatotoxin and heapatocarcinogenic agent known to induce cancer in experimental animals (2) and Phenobarbital acts as a tumour promoter when administered together with a tumour initiating carcinogen like N-nitroso-diethylamine. Combination of DEN/PB has been used in several experimental models to screen the protective effects of various natural and synthetic compounds in animals. (3).Most of the research performed today focuses on the development of new drugs to treat cancer and cancer related alteration in biochemical parameters. One of the most attractive approaches to disease prevention or treatment involves the use of natural antioxidants to protect tissue against toxic injury.

Antioxidants act as a major defence mechanism by protecting the damages caused by free radicals. So drugs or products which contains antioxidants are getting increased focus in the last three decades for the prevention and treatment of complex diseases like, diabetes, Alzheimer's disease and cancer. This has attracted a great deal of research interest in natural antioxidants. Many investigations are now being carried out to discover naturally

occurring compounds,which can suppress or prevent the process of carcinogenesis and its biochemical alterations (4).

Polyalthia longifolia (family: annonaceae) is a lofty evergreen tree found in India and Sri Lanka, commonly planted for its effectiveness in alleviating noise pollution. In India, the seeds and bark of the plant are used as febrifuge in the Balasore district of Orissa (5). The extract of stem bark of the plant and the alkaloids isolated from it has been reported for antibacterial and antifungal activities(Hasan et al.,1988). Its aqueous extract stimulates the isolated ileum and uterus, depresses heart rate, decrease blood pressure and respiration rate in experimental ani-mals(6) The crude extracts of the seeds of the plant have also showed remarkable antibacterial activities and plants of annonaceae family contain antitumor and anti-cancer active principles(7). In addition to this, Methanolic fraction of *Polyalthiya longifolia* leaves showed anticancer potency against DEN induced hepatocellualr carcinoma. More over the various parts of *Polyalthiya longifolia* yielded more than twenty anticytotoxic compounds along with flavounoids, triterpinoids and phenolic compounds.In view of the above, The present study was aimed to study the effects of methanolic extract of *Polyalthiya longifolia* fruits on DEN/PB induced Hepatocellular damage with special reference to lipid profile and antioxidant status.

MATERIALS AND METHODS

Chemicals

N-nitrosodiethylamine and Phenobarbital were procured from Sigma-Aldrich, India. The assay kits for various lipid profile test were purchased from nicholas laboratories. Chemicals used in this study,1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, India, ascorbic acid, phosphoric acid, naphthyl etthylene diamine dihydrochloride, are obtained from Sd Fine chemicals Ltd, India, and potassium ferricyanide, trichloro acetic acid, sulfanilamide were obtained from Himedia, Laboratories Pvt-Ltd, India, Folin-Ciocalteu reagent, sodium nitroprusside obtained from Qualigens fine chemicals and all the reagents used in the study were analytical grade.

Plant material

Fruits of *Polyalthiya longifolia* were collected from Irumbulikurichi, Ariyalur district,Tamilnadu, India and authenticated by G.V.S Murthy, botanical survey of India (BSI),southern circle, Coimbatore, Tamilnadu, India (BSI/SC/5/23/11-12/Tech-1759).

Preparation of Polyalthiya longifolia methanolic extract

The shade dried and chopped fruits were powdered and passed through a 40-mesh sieve then extracted with methanol by using Soxhlet apparatus. The solvent from the methanolic extract was completely removed and concentrated to dryness at 40°c under reduced pressure in a rotary vacuum evaporator. The *Polyalthiya longifolia fruits* yielded brown semi-solid residue of methanolic extract, weighing 9.0% w/w with respective to the dried starting material.

Phytochemical screening

The preliminary Phytochemical screening of crude extract of *Polyalthiya longifolia* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols (8).

Experimental animals

The experiments were carried out on adult male albino rats (150-180g). They were housed in a quite temperature of 25 ± 1^0C and relative humidity of 45-55%.They were fed with standard rat feed with water ad libitum, except during the test period. Each group consists of a 6 animals/dose and the experimental protocols were approved by institutional animal ethics committee (IAEC-KMCRET/Pharm/06/2011) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

Toxicity Evaluation Test

Acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; acute oral toxicity acute toxic class method. Wistar rats (n= 3) of either sex were selected by a random sampling technique for the acute toxicity study. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Extract was administrated orally in increasing dose up to 2000mg/kg.

Experimental Design

The rats were divided into five groups and each group consisting of six animals.

1. Groups I - Normal control-Treated with Normal saline (0.9%)

2. Group II - Untreated- *DEN* [200 mg/kg + PB 0.05% for 14 weeks]
3. Group III - HCC bearing rats+ MEPL Extract (200 mg/kg) for 28 Days
4. Group IV - HCC bearing rats+ MEPL Extract (400 mg/kg) for 28 Days
5. Group V - Sylimarin 200 mg/kg

After 2 weeks of DEN (200 mg/kg/i.p) administration, Phenobarbital (PB) were given to promote hepatocellular carcinoma for up to 14 successive weeks. Extracts were given post orally for 28 days to hepatocellular carcinoma bearing rats. After the experimental period, the animals were sacrificed by cervical decapitation under ether anaesthesia following animal ethics guidelines. Liver and kidney samples were collected for further biochemical analysis.

Biochemical parameters

Blood and serum obtained from the animals were used for the estimation of glucose, urea (9), creatinine (10) and lipid profile parameters. The blood glucose level was measured using a digital one touch glucometer (Abbott Diabetes care Inc., Alameda, USA). A drop of blood was placed on one side of a test strip that was inserted in the glucometer. The glucose level was displayed on the screen within 20s. Free fatty acid (FFA) was measured by using standard methods (11).Estimation of serum TC, TG, HDL, was performed using standard assay kits (Nicholas India Pvt. Ltd.) with semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd.). VLDL, LDL cholesterol was calculated by using formula.

VLDL = Triglycerides / 5

LDL = Total cholesterol – (HDL+VLDL)

Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of methanolic extract of *Polyalthiya longifolia* fruits was measured by the decrease in the absorbance of methanolic solution of DPPH and this activity was measured by spectrophotometric method. 1mL of methanolic solution of extract of MEPL at various concentrations (25,50,100,200,400 and 800 µg/mL) were mixed with 1mL of methanolic solution of DPPH (100µM). Similarly a 1mL methanolic solution of ascorbic acid (100 µg/mL) was mixed with 1mL of DPPH solution. A mixture of 1mL of methanol and 1mL of methnolic solution of DPPH (100 µM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and absorbance was measured at 517 nm (12). The experiments were performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\text{Scavenging \%} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5ml of each extract solutions of different concentrations (25,50,100,200,400 and 800 µg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours. In an identical manner 5mL of ascorbic acid solution (100 µg/mL) in standard phosphate buffer solution (pH 7.4) was also incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4). Control experiments without the extract but with equivalent amount of buffer were also conducted. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (Sulphanilamide 1%, O-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm (13).From the absorbance the percent scavenging activity was calculated. The experiments were performed in triplicate and scavenging activity was expressed as percentage of inhibition.

Reducing power

2.5mL of solutions of different concentrations of the extract (25,50,100,200,400 and 800 µg/mL) in standard phosphate buffer solution (pH 6.6) were incubated with 2.5mL of potassium ferri cyanide solution (1% w/v) at 50°C for 20 min. In an identical manner solution of ascorbic acid (100 µg/mL) was also incubated. After incubation, 2.5mL of 10% trichloro acetic acid solution was added to each tube and the mixture was centrifuged at 650 rpm for 10 minutes. 5mL of the upper layer solution was mixed with 5mL of deionised water and 1mL of ferric chloride solution (1%w/v) and the absorbance was measured at 700 nm (14).

Statistical analysis

Results are expressed as mean±S.E.M. The significance of the difference in the responses of treatment groups in comparison to the control was determined by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. p<0.05 was considered statistically significant.

RESULTS

Phytochemical and toxicity studies

Preliminary phytochemical screening of the plant showed the presence of Alkaloids, terpinoids, flavonoids, glycosides, tannins and phenolics. Results of acute toxicity studies indicates, the behaviour of the MEPL treated rats appeared normal. No toxic effect was reported at doses up to 3-5 times of effective dose of the methanol extract and there was no death in any of the groups which received methanolic extract of *Polyalthiya longifolia* fruits.

Blood glucose

DEN treated animals showed significantly decreased blood glucose levels (48.26±5.12) when compared to normal control (72.67±4.21) groups whereas treatment with MEPL 200 mg/kg and 400 mg/kg showed significant improved level of blood glucose (67.39±4.34 **p<01.0 & 69.41±5.52, **p<0.01 respectively) when compared to control groups (Table 1).

Table 1.Effect of MEPL on blood glucose, urea and creatinine level

Groups	Dose mg/kg	Units mg/ml		
		Glucose	Urea	Creatinine
Control	NS 0.9%	72.67±4.21	28.35±1.28	3.7±0.28
DEN + PB	200+0.05	48.26±5.12	23.45±0.82	1.2±0.82
DEN+ MEPL	200	67.39±4.34**	19.17±2.35*	1.6±0.27*
DEN+ MEPL	400	69.41±5.52**	25.57±2.43**	2.47±2.23**
DEN+ Sylimarin	200	67.38±5.31**	30.57±4.23**	2.57±4.23**

*Values are mean ±SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01 when compared to DEN treated groups*

Urea and Creatinine level

As depicted in Table 1.the urea and creatinine levels were decreased in DEN received group 2 animals. Administration of MEPL (200,400 mg/kg) showed significant increase in the urea (p<0.05) and creatinine (p<0.05) levels in group 3 and group 4 animals when compared to DEN treated groups (Table 1).

Lipid profile

DEN induced hepatocarcinogenic rats showed hypercholesterolemia (*p<0.05), hypertri-glyceridemia (**p< 0.01), increase in the free fatty acid (*p<0.05) , LDL (*p<0.05), VLDL(**p<0.01) and decrease in HDL(*p<0.05) as compared to the control rats. Whereas MEPL (200,400 mg/kg) treated rats showed considerably low levels of cholesterol, triglycerides, free fatty acids, LDL, VLDL and an marked increase in the HDL cholesterol as compared to the group 2 rats received DEN/PB.(Table 2,3)

Table 2.Effect of MEPL on serum lipid profile of DEN/PB induced hepatocellular carcinoma rats.

Treatment	Dose (mg/kg)	Lipid profiles (mg/dl)	
		TC	TG
Control	NS 0.9%	82.33 ± 0.55	74.32±1.43
DEN+PB	200+0.05	110.34±1.36*	145.83±1.86**
DEN+MEPL	200	105.49±1.94*	99.47±0.75**
DEN+MEPL	400	88.53±0.73**	88.39±0.63**
DEN+Sylimarin	200	80.64±0.69**	82.89±2.24**

*Values are mean ±SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01*
Sylimarin,MEPL treated groups were compared with DEN treated groups.

Table 3.Effect of MEPL on serum lipid profile of DEN/PB induced hepatocellular carcinoma rats

Treatment	Dose (mg/kg)	Lipid profiles (mg/dl)			
		LDL	HDL	VLDL	FFA
Control	NS 0.9%	54.79±1.60	46.38 ± 4.46	32.44±1.10	82.27±0.79
DEN+PB	200+0.05	87.62±.10*	43.66 ± 1.32	85.38±1.61***	120.31±1.61*
DEN+MEPL	200	76.41±0.79**	50.76 ± 0.78	66.71±1.62**	96.19±1.82**
DEN+MEPL	400	61.39±1.22**	57.28±0.80**	51.24±0.8**	85.00±1.48**
DEN+Sylimarin	200	50.43±1.73**	52.17± 0.81**	43.03±0.95**	89.05±1.51**

*Values are mean ±SEM for six animals, Values are statistically significant at *p<0.05, **p<0.01*
Sylimarin,MEPL treated groups were compared with DEN treated groups.

Antioxidant activity

Results from our invitro antioxidant activity in rats was revealed that MEPL scavenged free radicals in a concentration dependent manner in the models studied. Maximum percentage inhibition of DPPH radicals by the extract was about 62% at 800 µg/mL concentration whereas standard drug ascorbic acid showed about 81% inhibition of the DPPH radicals at 200µg/mL(Table.4).In the nitric oxide radical scavenging model, the maximum percentage inhibition of nitric oxide radicals by MEPL about 56% at 800µg/mL (Table 4.)Whereas ascorbic acid at 200µg showed about 62% inhibition and the reducing power of MEPL was dose dependent. The maximum absorbance of MEPL at 800µg/mL is comparable with ascorbic acid 100µg/mL (Table 4).

Table 4.Invitro Antioxidant activity of Methnolic extracts of *Polyalthiya longifolia* fruits.

Sample (µg/mL^{-1})	% Inhibition (Mean± SEM) Absorbance (Mean± SEM)		
	DPPH	NO	Reducing Power
MEPL 800	68.35±1.47	59.54±0.33	0.77±0.03
MEPL 400	61.59±1.63	51.63±0.49	0.77±0.05
MEPL 200	57.59±1.63	49.63±0.49	0.72±0.05
MEPL 100	46.48±1.22	31.65±0.31	0.66±0.08
MEPL 50	24.67±1.31	19.52±0.68	0.36±0.06
MEPL 25	19.51±1.49	09.37±0.19	0.14±0.005
Ascorbic acid 200	82.39±1.61	72.23±0.63	1.25±0.37
Ascorbic acid 100	68.37±2.34	39.36±0.22	0.79±0.06
Ascorbic acid 50	48.57±2.48	29.11±0.71	0.65±0.09
Ascorbic acid 25	35.34±2.56	19.72±0.83	0.59±0.02

Results from our invitro antioxidant activity in rats were revealed that MEPL scavenged free radicals in a concentration dependent manner in the models studied. Maximum percentage inhibition of DPPH radicals by the extract was about 68% at 800 µg/mL concentration whereas standard drug ascorbic acid showed about 82% inhibition of the DPPH radicals at 200µg/mL (Table.4).

In the nitric oxide radical scavenging model, the maximum percentage inhibition of nitric oxide radicals by MEPL about 59% at 800µg/mL (Table3.)Whereas ascorbic acid at 200µg showed about 72% inhibition and the reducing power of MEPL was dose dependent. The maximum absorbance of MEPL at 800µg/mL is comparable with ascorbic acid 100µg/mL (Table 4).

DISCUSSION

Generally, for the survival of cancer cells requires more amount of glucose as energy. Results from our study shows, glucose levels were decreased in DEN treated group II rats when compared with the normal control group which indicates the dysfunction of liver in DEN treated groups and initiation of cell proliferation. Treatment with MEPL(200,400 mg/kg.p.o) in hepatocellular bearing rats further decreased the glucose levels as compared to DEN received groups, making the liver cell deprived of glucose for energy production and utilization which indirectly indicates its chemo preventive action for the treatment of hepatocellular carcinoma

Additionally, significant reduction in the urea levels in DEN treated group II animals could be attributed to the declining function of the liver which is the primary site for the synthesis of urea. Reports from earlier studies shows, decrease in urea levels were noticed in liver dysfunction including hepatitis and cirrhosis (15).In addition to this, available datas also gives evidence that, significant reduction in blood urea levels in hepatic injury may be due to failure of the liver to convert amino acids and ammonia to urea (16).

In the present study, marked elevation of serum lipid profile and decreased levels of blood glucose in DEN treated group II animals clearly indicates the hepatocellular damage. Available datas from earlier studies shows, increased accumulation of serum cholesterol, triglycerides and free fatty acids during DEN/PB induced hepatocellular damage in rats (17). Reports from our study also revealed the significant increase in serum cholesterol, triglycerides and free fatty acids, LDL and VLDL in DEN treated group. The decreased levels of HDL and a significant increase in the levels of LDL and VLDL observed in the DEN/PB rats indicate clearly the hyperlipidaemic conditions caused by carcinogens like DEN. Interestingly, administration of MEPL (200,400 mg/kg.p.o) significantly decreased the elevated levels of TC,TG,FFA,LDL,VLDL and significantly increased the level of HDL in the group 3 and group 4 rats which gives an indication about the beneficial effects of administration of methanolic effect of *Polyalthiya longifolia*.

.

Additionally we have employed, The DPPH radical scavenging and nitric oxide radical scavenging method for direct measurement of radical scavenging and reducing power methods for indirect antioxidant measurement activity

of the extract.(18).Obtained results from our study indicates, the definite scavenging activity of the methanolic extract of towards DPPH radicals in comparison with ascorbic acid.

Nitric oxide is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However excess production of nitric oxide is associated with several diseases (19,20). Administration of Methanolic extract of *Polyalthiya longifolia* has demonstrated dose dependent radical scavenging activity against NO free radicals. So MEPL demonstrated good radical quenching activity against both DPPH and the nitric oxide radicals. In reducing power method, MEPL demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. In all the methods, scavenging effect of MEPL increases with increasing concentration and maximum antioxidant activity was found at the dose of 800 µg/mL and above of MEPL .

In summary, thus our findings demonstrate that MEPL has altered effect in lipid profile of animals with hepatocellular damage induced by DEN/PB, which is evidenced by the reduction of TC, TG, LDL-C, VLDL-C and increased HDL-C. Also results from our antioxidant study indicates, the definite radical quenching activity of the extract towards DPPH radicals, NO free radicals in comparison with ascorbic acid and in reducing power method, MEPL demonstrated dose dependent antioxidant activity comparable with ascorbic acid. Moreover results from phytochemiclal analysis of *Polyalthiya longifolia* fruits indicated the presence of flavonoids , phenolic compounds. In addition to this, in acute toxicity study, no death even with 3-5 times of effective dose indicating high margin of safety. Thus, it clearly indicates that *Polyalthiya longifolia* might be beneficial in attenuating the elevated biochemical parameters against DEN/PB induced hepatocellular damage in rats through its antioxidant property.

REFERENCES

[1] K.Okuda, *J Hepatol*.,**2000**,32,225.
[2] M.A.Pereira, S.L. Herren Freund,A.L.Britt,M.M.Khoury.*J Nat Cancer*,**1984**,72,741.
[3] T.Watanaben, S.Sugie,K, Okamoto,**2001**,*J Cancer Res*.,92,603.
[4] T.P.A.Devasagayam,J.C Tilak,K.K.*J Assoc Phys*.,**2004**,52,794–804.
[5] K.Raghunathan,M.K.Mitra MK, Central council for research in Ayurveda and Siddha,**1982**,1,127–139.
[6] G.Achari,S.V Lal,*Ind. Pharm*.**1952**,8,538–545.
[7] S.K.Chakrabarti, B.Mukherjee *J. Res. Ind. Med*., **1968**,3,84– 85.
[8] C.K.Kokat,A.P.Purohit,S.B.Gokhale.Text Book of Pharmacognosy. 4th Edn.CBS Publishers, **2000**,593-597.
[9] S.Natelson,M.L.Scott,C. Beffa C, *Am J Clin Pathol*.,**1951**,51,275.
[10] J.Broad ,J.H.Sirota, *J Clin Invest*.,**1948**,27,645.
[11] W.T.Hron ,L.A. Menahan LA, *J Lipid Res*.,**1981**,122,377.
[12] M.S.Blois MS,*Nature*.,**1958**,181,1199-1200.
[13] L.C.Green,D.A.Wagner,J.Glogowski,P.L.Skipper,*Anal Biochem*.,**1982**,126,131-138.
[14] M.Oyaizu,*Japanese J Nutr*.,**1986**,44,307-315.
[15] R.Anandan,M.S.Priya,K.P. Devi,T.Devaki T.*Med Sci Res*.,**1999**,27:127.
[16] R.T.Schmike,*J Biol Chem*.,**1992**,237,469.
[17] J.Jiang ,P.Nilsson Ehle,N.Xu,*Lipids Health Dis*.,**2006**,5:4.
[18] H.C.Wu,H.M.Chen,C.Y Shiau,*Food Res Int*.**2003**,36,949-957.
[19] R.Gibanananda, A.H.Sayed,*Indian J Exp Biol*.,**2002**,40,1213-1232.
[20] A.Ialenti,S.Moncada,M.Di Rosa, *Brit. J Pharmacol*.,**1993**,110,701-706.