ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Hepatoprotective, Antioxidant and Anticancer Properties of Saponins of *Momordica cymbalaria* in N-nitrosodiethylamine induced Hepatocellular Carcinoma in Albino Rats.

Maulik M Patel[1]*, Vamseedhar Annam[2], Sowmya MD[3], Nalinimohan C[2], and Raghunatha GV[3].

[1]Department of Pharmacology, Mamata Medical College, Khammam, Telangana, India.
[2]Department of Pathology, Mamata Medical College, Khammam, Telangana, India.
[3] Department of Dhravyaguna, Acharya Ayurvedic Medical College, Bangalore, Karnataka, India.

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the major causes of mortality and morbidity worldwide. Previous reports have emphasized the role of various chemotherapeutic drugs in HCC. Also there was one documented report of antioxidant herb, which has shown hepatoprotective action. The antioxidant and hepatoprotective properties of saponins of *Momordica cymbalaria* are well known; hence we evaluated the anticancer properties in N-Nitrosodiethylamine (DEN) induced HCC. Also we compared the efficacy of saponins of *Momordica cymbalaria* with standard treatment for HCC. The Induced rats treated with saponins of *Momordica cymbalaria* and doxorubocin showed significant reduction (but were above the upper limit of Normal group) of liver function tests when compared to induced group which had higher levels. The saponins of *Momordica cymbalaria* significantly increased the antioxidant levels and decreased the levels of oxidants when compared to induced Group. Histopathology of liver in treated groups with saponins of *Momordica Cymbalaria* showed tumor cell necrosis and antitumor angiogenesis while doxorubicin treated only showed necrosis of malignant cells. The saponins of *Momordica cymbalaria* possess hepatoprotective, antioxidant and anticancer properties. Also, it has better efficacy compared to doxorubicin as standard treatment.

Keywords: Saponins, *Momordica cymbalaria*, DEN, Hepatocellular Carcinoma.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem of worldwide significance, being the sixth most common cancer and the third leading cause of cancer-related death[1,2]. In cancer cells, oxidative stress induces a cellular redox imbalance which may be related to oncogenic stimulation. Permanent modification of genetic material resulting from "oxidative damage" incidents represents the first step involved in mutagenesis and carcinogenesis. ROS-induced DNA damage involves single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNAcross-links. DNA damage, mutations, and altered gene expression are thus all key players in the process of carcinogenesis [3, 4]. Thus use of antioxidants in the treatment could be beneficial in cancer. The anticancer drug like doxorubicin commonly used for treatment of HCC induces high oxidative stress in rat liver. Hence, antioxidants in treatment of HCC may be beneficial. It is well known that angiogenesis is essential for the survival, growth, and metastasis of tumor cells [5, 6]. Also intrahepatic metastasis is a critical factor leading to the advance of recurrence of advanced HCC. Hence, there is much interest in inhibiting angiogenesis as a treatment strategy in HCC.

Animal experimental models are particularly useful for the study of cancer in humans. As experimental model of human HCC, we used rats treated with DEN which induces poor, moderate and well differentiated forms of HCCs with histological features similar to those of the human tumors [7].

In recent years, there has been considerable emphasis on the identification of plant products with anticancer, hepatoprotective and antioxidant property in treatment of HCC. The medicinal value of the chosen plant *Momordica cymbalaria* (MC) has been worked out related to antioxidant and hepatoprotective property [8, 9]. However, its therapeutic efficacy in HCC has not been evaluated. The plant MC belongs to the family Cucurbitaceae, originating in tropical regions of South India and South East Asia. MC Hoof. is commonly known as Kasarakayee (Andra Pradesh) or Karchikai (Kannada) or Athalakkai (Tamil) and Kakrol (India). The plant is traditionally used for the treatment of diabetes mellitus, rheumatism, ulcer, skin disease, anti-ovulatory, abortifacient, anti-implantation activity and diarrhoea. The fruit of this plant have been reported to possess hypoglycaemic, hypolipidemic, cardio protective, hepatoprotective, nephroprotective and antioxidant properties. The major nutrient constituents of MC are reported to contain high concentrations of calcium, vitamin C, beta carotene and Iron [10].

MATERIALS AND METHODS

Experimental Animals

Male Albino rats of Wistar strain approximately 12-14weeks young rats weighing 150-175grams were procured from National Institute of Nutrition, Hyderabad, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Plant collection and preparation of the extract

The fresh roots of MC was collected from Andhra Pradesh, India. The roots were isolated, chopped into small pieces and later dried under shade at room temperature for 10 days, and powdered by mixer grinder. The powdered sample was defatted by petroleum ether for 3 hours at 40˚c. After filtering the petroleum ether, the sample was extracted with methanol for 3 hours by mild heating. The methanol extract was concentrated and re-extracted with methanol and acetone (1:5v/v) [11].The precipitate obtained was dried under vacuum, which turned into whitish amorphous powder after complete drying. It was loaded on Merck silica gel-60(230-400mesh) column and eluted with chloroform-methanol-water (70:30:10) [12]. The first fraction collected was air dried at room temperature (28˚c) and the residue obtained was treated as pure saponins of momordica cymbalaria (SMC). The purity of the saponins isolated was analysed by thin layer chromatography using chloroform and methanol (7:3) as the solvent system [13].

Experimental Design

Body weights of the animals were recorded and they were divided into 4 groups (Table.1) of 6 animals. Animals were fasted for 24 hours before the study but had free access to water.

Table 1: Experimental Groups (n=24)

Group I (n=6)	Normal Group	Untreated	Normal Saline
Group II (n=6)	Induced Group	N-nitrosodiethylamine (DEN)	200mg/kg IP single dose
Group III (n=6)	Standard Group	Doxorubicin	2mg/kg/week IV for 45days after induction
Group IV (n=6)	Test Group	Saponins of *Momordica cymbalaria(SMC)*	175mg/kg/day oral for 45 days after induction

Biochemical Parameters

The Liver function tests and Oxidant Parameters were done after 36 hours in Group II and at the end of the experimental period (45 days) in group III and IV. The blood samples were collected from the retro orbital venous plexus & serum were separated and analyzed for various parameters like liver function tests parameters and oxidative stress parameters

The liver function tests included total bilirubin, direct bilirubin, serum glutamic oxaloacetic acid-transaminase (SGOT), serum glutamic phosphoacetic acid-transaminase (SGPT) and serum alkaline phosphatase (ALP) which was assayed in the serum by using Fully Automated Biochemistry Analyser (Robonik India Pvt. Ltd.). The oxidative stress parameters consisted of Malondialdehyde (MDA) levels and antioxidant enzymes. The liver homogenate was centrifuged at 10,000 xg at 00 for 20 minutes using Remi C-24 high speed cooling centrifuge and supernant was used for the assay of lipid peroxidation (MDA) [14], endogenous antioxidant enzymes, reduced glutathione (GSH) [15], catalase (CAT) [16], and super oxide dismutase (SOD) [17].

Histopathology examination

Liver specimen was fixed in 10% neutral buffered formalin for histopathology study. The pieces of liver were processed in automated tissue processor and embedded in paraffin wax. Sections of about 4-6 µm were cut by semi-automated microtome and stained with hematoxylin and eosin for histopatholgy examination.

Statistical analysis

All results were expressed as mean ± standard error of the mean. Data analysis was achieved by one-way analysis of variance (ANOVA) and comparisons between the groups were done by student's t-test. P values <0.05 were considered significant.

RESULTS

Oxidative Stress parameters

Malondialdehyde (MDA) level was significantly ($p<0.001$) increased and the levels of GSH, CAT and SOD were significantly ($p<0.001$) decreased in DEN treated rats when compared with those of the animals in control group. Administering SMC (175mg/kg) in DEN administered rats significantly decreased MDA and increased the levels of GSH, CAT and SOD (Table.2). The results are well comparable with doxorubicin + DEN administered group.

Biochemical parameters

The activities of serum hepatic marker enzymes namely SGOT, SGPT and ALP showed a significant ($p<0.001$) increase in DEN treated rats as compared to normal group (Table.3). After administering SMC, there was significant ($p<0.001$) reduction in the levels of SGOT, SGPT and ALP in DEN treated rats as compared to the animals treated with DEN alone and standard group.

When compared to normal group the serum total and direct bilirubin were significantly ($p<0.001$) increased in DEN treated group (Tables.3) and the levels of both significantly decreased in SMC+ DEN and doxorubicin + DEN group as compared to DEN treated rats.

Table 2: Effect of DEN, Saponins of *Momordica cymbalaria* and doxorubicin on oxidative stress parameters

Groups	Treatment	SOD	CATALASE	GSH	MDA
I	Normal Group	6.54±0.2	7.32±0.12	12.41±0.08	5.44±0.10
II	Induced Group	2.52±0.02*	2.64±0.04*	6.73±0.08*	18.24±0.12*
III	Standard Group	5.25±0.12$^{\#}$	6.98±0.08$^{\#}$	10.34±0.06$^{\#}$	12.12±0.10$^{\#}$
IV	Test Group	9.46±0.02$^{*\#}$	8.73±0.03$^{*\#}$	14.88±0.02$^{*\#}$	4.64±0.08$^{*\#}$

Values are expressed as mean ± SEM, n = 6.
*P<0.0001 is considered statistically significant compared to Normal group.
$^{\#}$P<0.0001is considered statistically significant compared to DEN induced group.
*$^{\#}$P<0.0001 is considered statistically significant compared to Normal group and DEN induced Group

Table 3: Effect of DEN, saponins of *Momordica Cymbalaria* and doxorubicin on liver function test

Groups	Treatment	SGOT	SGPT	ALP	Total Bilirubin	Direct Bilirubin
I	Normal Group	38.14±0.53	37.72±1.31	59.86±1.21	0.70±0.05	0.76±0.03
II	Induced Group	73.5±1.2*	78.6±0.60*	104.5±1.5*	3.10±0.2*	1.12±0.1*
IV	Standard Group	48.4±0.8$^{\#}$	49.2±0.9$^{\#}$	79.6±1.1$^{\#}$	1.58±0.1$^{\#}$	0.91±0.02$^{\#}$
III	Test Group	41.42±0.2$^{\#}$	40.65±0.4$^{\#}$	64.41±1.0$^{\#}$	1.01±0.1$^{\#}$	0.84±0.1$^{\#}$

Values are expressed as mean ± SEM, n = 6.
**P<0.0001 is considered statistically significant compared to Normal group*
$^{\#}$*P<0.0001 is considered statistically significant compared to* DEN induced group

Histopathology observation

Histopathological study of liver from normal control group animals showed intact architecture of the liver (Figure 1). All the rats had HCC after treatment with DEN. The cancer cells were arranged diffusely with pleomorphic vesicular nucleus and moderate cytoplasm (Figure 2). Treatment with SMC (Figure 4) or doxorubicin (Figure 3) to DEN treated rats exhibited necrosis of cancer cells which had membrane blebbing and cytoplasmic vacuolization. In SMC treated rats, antiangiogenesis was observed.

ISSN: 0975-8585

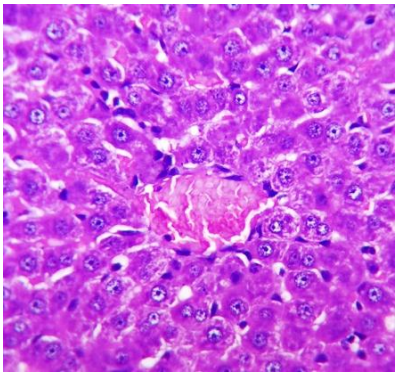

Figure 1: Normal control group with intact architecture

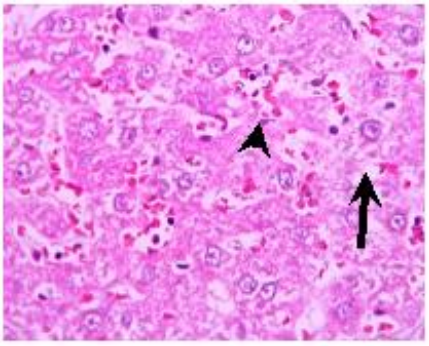

Figure 2: DEN induced group with HCC cells [Arrow] and increased blood vessels [Arrow Head]

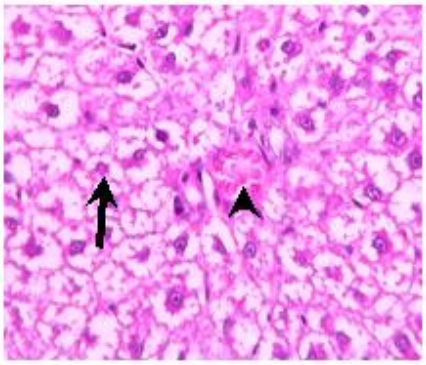

Figure 3: Doxorubicin treated group with HCC cell necrosis [Arrow] and intact blood vessels [Arrow Head]

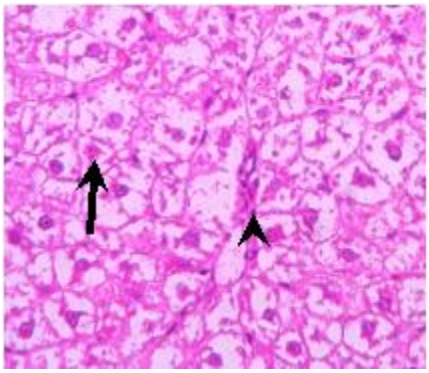

Figure 4: *Momordica Cymbalaria* treated group with HCC cell necrosis, apoptosis [Arrow] and disrupted blood vessels [Arrow Head]

DISCUSSION

HCC is one of the highly aggressive malignant neoplasm and presents with limited therapeutic options. Hepatocarcinogenesis induced by DEN is an ideal animal model to investigate liver tumor formation because it proceeds in stages similar to that of human liver cancer [18]. DEN metabolism induces depletion in the activities of antioxidant enzymes that can be owed to an enhanced free radical production. Excessive generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in lipid peroxidation (LPO), mutagenesis and carcinogenesis [19]. In the present study, there was significant reduction in antioxidant enzymes and increase in MDA levels in DEN induced rats. This might be attributed to increased tumor growth rate in DEN induced rats. In standard group, there was still increase in oxidants. The rate of increase in antioxidants after SMC administration suggested that there was scavenging of excessive free radicals in the body and thus process of carcinogenesis was hindered.

The biochemical marker enzymes of liver are more unique and changes in their activities reflect the effect of proliferation of cells with growth potential and its metabolic turnover. In cancer conditions, there will be a disturbance in the transport function carried out by cell organelles including hepatocytes, resulting in the leakage of enzymes due to altered permeability of plasma membrane, and thereby causing a decreased level of these marker enzymes in the cells and increased level in serum. The structural integrity of the cells has been reported to be damaged in toxicity induced animals and this results in cytoplasmic leakage of enzyme into the blood stream [20, 21]. In this study, increase in SGOT, SGPT and ALP levels upon DEN induction might due to disturbance in the transport function and the leakage of the enzyme. Like standard group, after administration of SMC, the enzyme levels were nearing to normal; probably the disturbance and enzyme leakage were rectified indicating hepatoprotective activity.

It is well known that angiogenesis is essential for the survival, growth, and metastasis of tumor cells [5, 6]. Angiogenesis, formation of new blood vessels from the existing vascular bed, is a complex multistep process. There is extracellular matrix remodeling and binding of angiogenic factors to specific endothelial cell (EC) receptors, which results in EC proliferation, invasion of basement membrane, migration, differentiation, and formation of new capillary tubes. Their anastomoses develop a vascular network [22, 23]. In our study, DEN treated group had increased blood vessels indicating angiogenesis while these blood vessels were disrupted in rats treated with SMC suggesting its antiangiogenic potential. In standard group the blood vessels remained intact suggesting no antiangiogenic role.

DEN induced animals showed altered morphological structures such as dysplastic nuclei, membrane changes with irregular cytoplasm. In addition to coagulative necrosis seen in standard group, apoptosis was also seen in SMC treated rats. The signs of stimulation of apoptosis were noted by the presence of liver cell with shrunken nucleus, condensed chromatin, membrane blebbing and formation of apoptotic bodies in SMC treated rats.

Therefore, in our study, SMC might render protection to macromolecules to avoid damage from xenobiotic such as DEN by maintaining the antioxidant, hepatoprotective, and antiangiogenic properties thereby exhibit anticancer activity during DEN induced liver cancer.

CONCLUSION

The results of the present study demonstrate that SMC increases antioxidant status, normalizes biochemical marker enzymes and antiangiogenic. Further, SMC provides evidence for induction of apoptosis and also necrosis. Thus the results of the present investigation have confirmed the efficacy of SMC as an effective chemotherapeutic agent.

REFERENCES

[1] Parkin DM, Bray F, Ferlay J and Pisani P. CA Cancer J Clin 2005; 55:74-108.
[2] Thomas MB, Zhu AX. J Clin Oncol 2005; 23: 2892-2899.
[3] Valko M, Izakovic M, Mazur M, Rhodes CJ and Telser J. Mol Cell Biochem 2004; 266: 37-56.
[4] Marnett LJ. Carcinogenesis 2000; 21: 361-370.
[5] Denekamp J. Br J Radiol 1993;66: 181-196.

[6] Fox SB, Gatter KC, Harris AL. J Pathol1996;179: 232-237.
[7] Di Stefano G, Fiume L, Bolondi L, Lanza M, Pariali M, Chieco P. Liver Int 2005;25:854–860.
[8] Prashanth, S.J., Suresh, D. and Maiya, P. Sadananda. Asian J Bio Sci 2013;8(1):107-116.
[9] Koneri R, Balaraman R, Firdous, Vinoth Kumar M. Pharmacologyonline 2008;1: 365-374.
[10] Jeyadevi R, Sivasudha A, Rameshkumar T, Sangeetha A, ArulAnanth B, Aseervatham GSB. Asian Pacific J Tropical Biomed 2012; S456-461.
[11] Takemoto DJ, Dunford C, McMurray MM. Toxicon 1982; 20:593–599.
[12] Zhu ZJ, Zhong ZC, Luo ZY, Xiao ZY. Yao Hsueh Hsueh Pao 1990;25(12):898–903.
[13] Sari H, Sirpa OK, Marina I, Hannu M. J Agri Food Chem 1999; 47(6):2274-79.
[14] Wilbur KM, Bernheim F, Shapiro OW. Arch Biochem 1949;24: 305-310.
[15] Ellman GL. Arch Biochem Biophys 1959; 82:70-77.
[16] Kono Y. Arch Biochem Biophys 1978;186:189-195.
[17] Hugo EB. Oxidoreductase activity on groups other than CHOH: Catalase. In: Colowick SP, Kaplan NO, Packer L ed. Methods in Enzymalogy London: Academic Press 1984;105: 121-125.
[18] Peto R, Gary R, Brantom P, Grasso P. Cancer Res 1991;51: 6452–6469.
[19] Hristozov D, Gadjeva V, Vlaykova T, Dimitrov G. Arch Physiol Biochem 2001;109: 331–336.
[20] Mc-Intrye N, Rosalki S. Biochemical investigation in the management of liver diseases. In: Prieto J, Rodes J, Shafritz DA, editors. Hepatobiliary Diseases. Berlin:Springer-Verlag 1992. pp. 39–71.
[21] Kamdem L, Siest G, Magdalou J. Biochem Pharmacol 1982;31:3057–62.
[22] Hanahan D, Folkman J. Cell 1996; 86: 353-364.
[23] Yanase T, Tamura M, Fulita K, Kodama S, Tanaka K. Cancer Res 1993; 53: 2566-2570.