International Journal of Pharmacological Research www.ssjournals.com
ISSN: 2277-3312 Journal DOI:10.7439/ijpr

Protective role of *Momordica cymbalaria* in diethyl nitrosamine induced hepatocellular carcinoma

Nagarathana PKM, Sanjib Kumar Yadav*, P. Satish and Dipendra Sah

Karnataka College of Pharmacy, Thirumenahalli Bangalore-560064, India

Corresponding author*
Sanjib Kumar Yadav
PG Scholar,
Karnataka College of Pharmacy,
Thirumenahalli Bangalore-560064
E-mail: yadavsanjibkumar@gamil.com

Abstract
Objective: The objective of this study was to evaluate the protective role of *Momordica cymbalaria* against N-nitroso diethylamine(DEN) induced hepatocelllular carcinoma.
Method: The protective effect of the plant extracts were evaluated by inducing Hepatocellular carcinoma in rat by giving single dose of 200mg/kg of N-nitroso diethylamine effect of DEN is potentiated by administering Phenobarbital in water. Various serum biochemical and histo-pathological studies were done to determine the effect of plant extract on hepatocellular carcinoma. The levels of GSH, SOD, LPO and CAT and activities of antioxidant enzymes were assessed in the haemolysate and liver of experimental animals to evaluate the antioxidant status.
Result: The results of the biochemical estimations demonstrated that the hepatocellular carcinoma was successfully induced by the DEN whereas the effect of DEN was reversed by the administration of the bark extracts. The antioxidant effect of the plant extracts were proved by estimating various parameters of the liver tissue homogenate. All these results indicate that the methanolic extract of *Momordica cymbalaria* have chemopreventive effect against DEN induced hepatocellular carcinoma.
Conclusion: the present results suggest that saponin of Momordica cymbalaria exerts protective effect by modulating the antioxidant status during DEN induced hepatocarcinogenesis.
Keywords: Antioxidant, N-nitroso diethylamine(DEN), Hepatotoprotective, *Momordica cymbalaria*.

1. Introduction

Hepatocellular carcinoma is the sixth most common cancer among malignant tumors and the third most common cause of cancer related deaths.[1] It has been accepted men show higher incidence of liver tumor than women [2], with men: women ratios usually averaging 2: 1.

Among sex hormones, estrogens were associated with decreased incidence of hepatocellular carcinoma [3,4] Actually, our previous study reported that estrogen treatment inhibited diethylnitrosamine(DEN)-induced hepatic tumors associated alteration of ERα loss [5]. However, chronic use of estrogens was associated with an increased risk of developing liver tumors in humans and some synthetic estrogens might act as cancer promoting agents [6,7]. And several human studies have reported an increased risk of developing malignant liver tumors as well as benign liver ones in women using oral contraceptives [8]. It is well known that chronic alcohol consumption has long been associated with progressive liver disease toward the development of hepatic cirrhosis and the progression to HCC, especially in developed countries [9-11]. Chronic ethanol feeding can accelerate HCC progression in male mice than females in the setting of being initiated by DEN [12].

Momordica cymbalaria, Fenzl. (Family: Cucurbitaceae) is a tuberous perenninal with very slender scandent, branched, striate, pubescent or subglabrous stem, the whole plant is acrid *Momordica cymbalaria* (family: cucurbitaceae) is found in the western parts of India from Satara district in the north down to the Tinnvelly in the south. The root of *Momordica cymbalaria* has shown antidiabetic.[13,14] cardioprotective[15] antifertility[15,16], antihyperlipidemic[17], antiovulatory and abortification activities[18], hepatoprotective, analgesic, anti-inflammatory activities.[19,20] therefore planned to study the effects of saponins of *Momordica cymbalaria* against diethyl nitrosamine induced hepatocellular carcinoma.

2. Material and methods

2.1 Collection and authentication of plant material

The fresh roots of *Momordica cymbalaria* , fenzl. Were collected from Gadag district, Karnataka, identified by Dr. Sreenath, Department of Boatany, Bangalore University, Bangalore.

2.2 Preparation of extract

The roots of *Momordiaca cymbalaria* were isolated, chopped into small pieces and dried under shade at room temperature for seven days. The dried roots were powdered and 95% w/v methanolic extract was prepared by soxhet extraction method. The dried powdered fruits of *Momordica cymbalaria* (200gm) were extracted with 95% v/v methanol for 21 hours using soxhlet extractor. The combined extracts were concentrated at 40^0C to obtain dark brownish yellow residue.

2.3 Saponin isolation

The methanolic extracts of *Momordica cymbalaria* was dissolved in hot dissolved water and was partitioned between water saturated n-butanol and a water layer. The organic layer (n-butanolic layer) was separated and evaporated to get a residue. This n-butanolic residue was dissolved in methanol and was poured in diethyl ether to obtain a flocculent precipitate. This precipitate was separated by using a filter paper and was with excess of diethyl ether and dried to yield a crude fraction of saponins. The saponin mixture was dissolved in distilled water (SMC) and was used for the study.

2.4 Experimental animals

Albino wistar rats weighing 100-120g were purchased from Indian Institute of Science (IISC) Bangalore. The animals were housed in polypropylene cages maintained in controlled temperature (27 ± 2°C) and light cycle (12h light and 12 h dark) and fed with standard rat pellet diet (Amrut rat and mice feed, India) and water *ad libitum*. All the experimental procedures were on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

2.5 Experimental design [21-23]

The rats were divided into 4 groups each group consisting of six animals. Liver tumor was induced in group consisting 2,3 and 4 with single intraperitoneal injection of [IP injection is the injection of a substance into the peritoneum (body cavity). IP injection is more often applied to animals than to humans]. In general, it is preferred when large amounts of blood replacement fluids are needed, or when low blood pressure or other problems prevent the use of a suitable blood vessel for intravenous injection. DEN at a dose of 200mg/kg body weight in saline, two week after DEN administration the carcinogenic effect was promoted by 0.05% Phenobarbital, which was supplemented to experimental animal through drinking water up to 16 successive 3 months.

Group A (Control): Normal control animals treated with 1ml of 10% DMSO.

Group B (DEN+PB): animals induce for HCC with DEN (200 mg/kg single i.p. injection) and promoted with PB (0.05%) in basal diet daily for the entire experimental period of 3 months.

Group C(DEN+PB+ saponins of *Momordic cymbalaria*): Animals induced for HCC with DEN (200 mg/kg b.w. single i.p. injection) promoted with PB (0.05%) in basal diet daily and administered orally through gavages with daily for saponins of *Momordica cymbalaria* (175 mg/kg .po/day) the entire experimental period of 3 months.

Group D (DEN+PB+Doxorubacin): Animals induced for HCC with DEN (200 mg/kg b.w. single i.p. injection) promoted with PB (0.05%) in basal diet daily and administered i.p. injection with daily for doxyrubacin[54] (2mg /kg i.p./day)

At the end of the experiment, animals were fasted overnight and killed by cervical decapitation. Blood was collected; serum was separated out and used for the assay of marker enzymes. The liver was immediately removed, weighed and suspended in cold saline. A small portion of liver was fixed in 10% formalin for histopathological studies.

2.6 Serum biochemical estimations:

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), SGOT, and SGPT were assayed using standard kits (SPAN India Ltd, Surat). The results were expressed as units/liter (IU/L).The levels of total protein, total billirubin, cholesterol and triglyceride were estimated in the serum using standard commercial kits from (SPAN India Ltd, Surat, India).

2.7 Assessment of hepatic Oxidative Stress marker enzymes

The liver homogenate was centrifuged at 10,000 xg at 0^0C for 20 minutes using Remi C-24 high speed cooling centrifuge and superatant was used for the assay of lipid peroxidation (malondialdehyde content), endogenous antioxidant enzymes, reduced glutathione (GSH) catalase (CAT), and super oxide dismutase (SOD).

2.7.1 Estimation of lipid peroxidation activity

The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) as described by Wilbur14. Briefly 1 ml of trichloroacetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 0.67% were added to 2 ml of homogenate supernant. The absorbance of the mixture was recorded at 530 nm and the values were expressed as ηM of MDA formed /mg of protein

2.7.2 Estimation of reduced glutathione activity

Reduced glutathione (GSH) in the rat hearts was assayed by the method described by Ellman15.Briefly 0.02ml of

the homogenate supernant was added to 3ml of Ellman reagent. The changes in absorbance were read at 412 ηm. The amount of glutathione was expressed as µg of GSH/mg protein.

2.7.3 Estimation of SOD activity

The level of SOD was measured by the method of Kono16. Briefly 1.3 ml of solution A (0.1 nM EDTA containing 50 nM Na2CO3, pH 10.3), 0.5 ml of solution B (90 M NBTnitro blue tetrazolium dye) and 0.1 ml of solution C (20mM Hydroxylamine hydrochloride, pH6.0) were mixed and the rate of NBT reduction was recorded at 560ηm. SOD activity was expressed as unit/mg protein.

2.7.4 Estimation of Catalase activity

Catalase activity was estimated by determining the decomposition of H_2O_2 at 240 ηm in an assay mixture containing phosphate buffer as described by Hug O E Aebi[17]. The activity was expressed in units as µM of H_2O_2 consumed per min/mg of protein. Histopathological examination: Liver pieces were preserved in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax. Sections of about 4-6 µm were made and stained with hematoxylin and eosin and photographed.

2.8 Statistical analysis

The statistical analysis were carried out by one-way analysis of variance (ANOVA), followed by Tukey Kramer multiple comparison post-test. P values

3. Results and Discussion

3.1 Serum biochemical parameters:

The activities of serum hepatic marker enzymes namely ALP, SGOT and SGPT showed a significant ($p< 0.001$) increase in DEN (200mg/kg.i.p/single dose) treated rats as compared to control group (Table 1). Administration of saponin of *Momordica cymbalaria* (175mg/kg.po/day/90days) significantly ($p< 0.001$) reduced the levels of ALP, SGOT and SGPT in DEN treated rats as compared to the normal DEN control rats. Administration of Doxorubicin (2mg/kg ip/day/90days) significantly ($p< 0.001$)) reduced the levels of ALP, SGOT and SGPT in DEN treated rats as compared to the normal DEN control rats.

When compared to control group the total billirubin was significantly ($p< 0.001$)) increased in DEN (200mg/kg.ip/single dose) treated group as compared to control group (Tables 1). Administration of saponin of *Momordica cymbalaria* (175mg/kg.po/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the total billirubin when compared to the normal DEN control rats. Administration of Doxorubicin (2mg/kg ip/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the total billirubin when compared to the normal DEN control rats.

When compared to control group the direct billirubin was significantly ($p< 0.001$)) increased in DEN (200mg/kg.ip/single dose) treated group as compared to control group (Tables 1). Administration of saponin of *Momordica cymbalaria* (175mg/kg.po/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the direct billirubin when compared to the normal DEN control rats. Administration of Doxorubicin (2mg/kg ip/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the direct billirubin when compared to the normal DEN control rats.

3.2 Hepatic Oxidative Stress parameters:

When compared to control group the SOD, CAT, GSH and LPO was significantly ($p< 0.001$)) increased in DEN (200mg/kg.ip/single dose) treated group as compared to control group (Tables 1). Administration of saponin of *Momordica cymbalaria* (175mg/kg.po/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the SOD, CAT GSH and LPO when compared to the normal DEN control rats. Administration of Doxorubicin (2mg/kg ip/day/90days) to DEN rats significantly ($p< 0.001$) reduced the level of the SOD, CAT, GSH and LPO when compared to the normal DEN control rats.

Table No 1: Effect of saponin of *Momordica cymbalaria* (175mg/kg.po/day/90days) and Doxorubicin (2mg/kgip/day/90days) on liver function test (SGPT, SGOT, ALP, Total bilirubin and Direct bilirubin)

Groups	SGOT	SGPT	ALP	Total bilirubin	Direct bilirubin
Normal control	24.14±0.8	24.72±1.5	47.86±1.4	0.3871±0.1	0.2417±0.1
DEN(200mg/kg.ip./single dose)	66.9±1.8***	64.6±0.64***	106.5±1.7###	3.041±0.1***	1.105±0.1***
Doxorubicin (2mg/kg ip/day/90 days	42.5±1.3###	44.67±1.5###	74.43±1.0###	1.213±0.1###	0.6533±0.1###
Saponins of *Momordica cymbalaria* (175mg/kg.po/day/90 days)	49.4±1.3###	47.2±0.9###	89.6±1.2###	1.640±0.1	0.8698±0.1###

Values are expressed as mean ± SEM, n=6

*** ($p< 0.001$) is considered statistically significant compared to Normal control,

($p< 0.001$ is considered statistically significant compared to DEN control.

Table 2: Effect of *Momordica cymbalaria* (175mg/kg.po/day/90days) and Doxorubicin (2mg/kg ip/90 days) on oxidative stress parameters (SOD, CAT, GSH, LPO) in liver tissue.

Groups	SOD	CATALASE	GSH	LPO
DMSO (1ml of 10%)	6.808±0.4	8.753±0.1	11.40±0.1	6.415±0.1
DEN (200mg/kg.ip/single dose)	1.497±0.02***	2.236±0.04***	5.688±0.1***	12.79±0.1***
Doxorubicin (2mg/kg ip./day/90days)	5.75±0.1###	7.081±0.06###	9.618±0.1###	4.691±0.1###
Saponin of *Momordica cymbalaria* (175mg/kgpo./day/90days)	6.26±0.05###	7.728±0.03###	10.19±0.08###	5.798±0.1###

Values are expressed as mean ± SEM, n=6
*** ($p< 0.001$) is considered statistically significant compared to Normal control,
($p< 0.001$ is considered statistically significant compared to DEN control.

3.3 Histopathology

(1) Normal Control

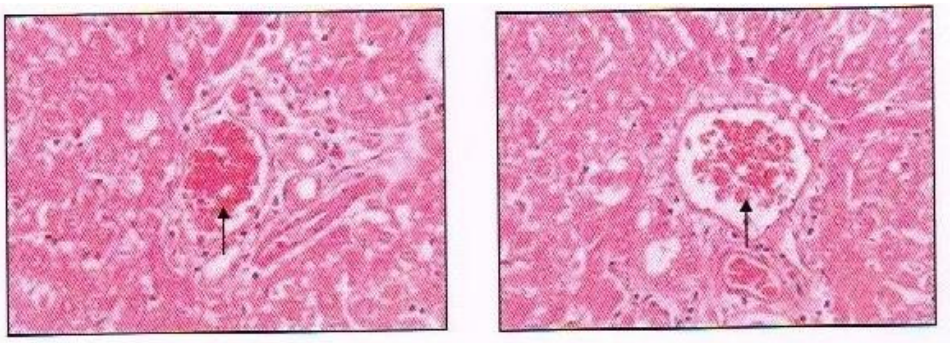

Figure 1 (a) & (b): few of the central veins congestion with intact sinusoid.

(2) DEN Control

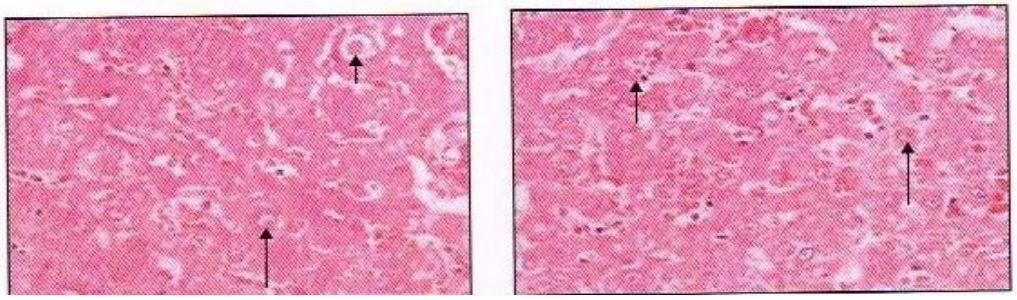

Figure 2(a): Malignant cell show nuclear pleomorphism with vascular nucleus and prominent eosinophilic nucleoli and atypical mitotic cells.

Figure 2(b): foeal areas of necrosis and hemorrhage midst these malignant cells.

(3) Standard (Doxorubacin)

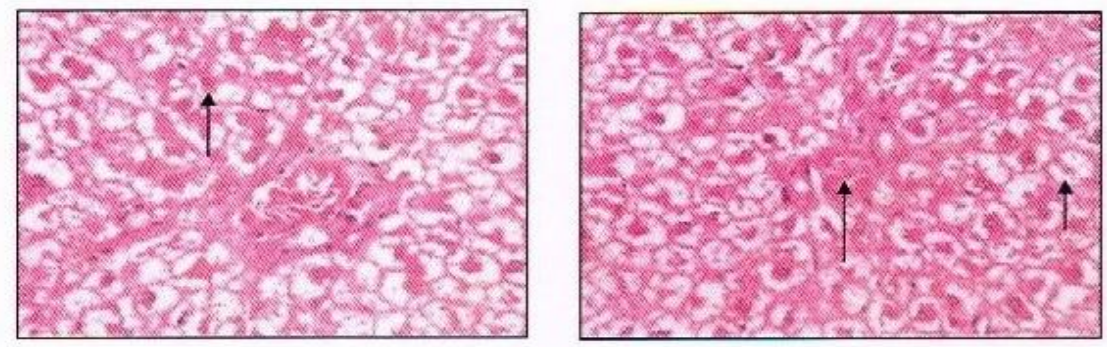

Figure 3(a): Hepatocyte with apoptotic change are seen.

Figure 3(b): Congested central vein with intact bile duct Are seen, most of hepatocytes show degenerati -ve changes consisting of disintegration nuceus and cytoplasm.

(4) Saponins (*Momordica cymbalaria)*

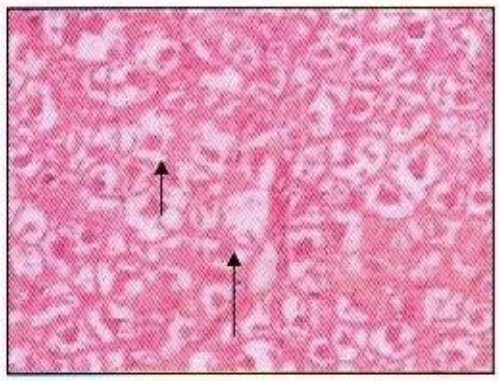

Figure 4(a): Hepatocytes with disintegration of nucleus and cytoplasm foecal are as Shown mononuclear inflammatory infiltration.

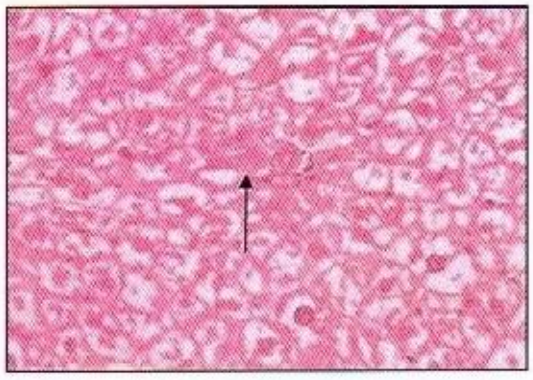

Figure 4(b): central veins appear disintegrated with loss of bile ducts.

4. Discussion

Hepatocellular carcinoma is a major problem not only in developed countries but also in most developing countries. It is induced by toxic industrial chemicals, air and air pollutants and also food additives and fungal toxins [24]. Since liver is the major site of metabolism of ingested materials, it is more susceptible to carcinogenic insult. Moreover, due to high tolerance of liver hepatocellular carcinoma is seldom detected at an early stage and once detected treatment has a poor prognosis in most cases.

HCC is one of the ten most common human cancers, with a worldwide incidence of over on million cases every year.[25] HCC a fatal malignancy represents 4% of all malignant tumors. N-Nitroso diethylamine is a widely occurring nitrosamine which is present in tobacco and various processed foods [26]. These nitroso compounds can also be formed in vivo in physiological conditions. N-Nitroso diethylamine primarily induces tumor of liver. It is widely accepted that activation of nitrosamines by cytochrome p450 enzyme to reactive electrophiles is required for their cytotoxic mutagenic and carcinogenic activity. Because of its relatively simple metabolic pathway and potent carcinogenic activity, DEN has found widespread use as an experimental model in the field of carcinogenesis and in chemoprevention. A single administration of DEN induced liver tumor is evidenced by the increase in liver weight, increased level of hepatic enzymes like SGPT, SGOT, ALP, Total bilirubin and direct bilirubin and increased level of SOD, CAT, GSH (oxidant enzymes) and morphological changes noted by physical examination. Elevated serum levels of SGOT, SGPT, ALP, total and direct bilirubin are indicative of poor hepatic function in DEN treated animals.

Treatment with the saponins of *Momordica cymbalaria* produced a significant reduction in tumor as revealed by reduction of morphological changes. In saponins of *Momordica cymbalaria* and doxorubicin treated group the activities of SOD, CAT and GSH were significantly increased when compared to DEN control.

The formation of Melondialdehyde is considered as an index of lipid peroxidation that causes cell injury. Elevation of lipid peroxides, as indicated by increased MDA was observed in DEN control group. Significant increase in LPO in carcinogenic process may be due to abnormal levels of reactive oxygen species.

In the present study Lipid peroxides level were significantly higher in DEN control group when compared to normal control group. The saponin of *Momordica cymbalaria* and doxorubicin treated group showed decreased LPO levels when compared to DEN control group.

Due to the free radical scavenging property the saponins of *Momordica cymbalaria* may cause reversal of oxidative stress induced by DEN and help in preventing the damage induced by free radicals.

5. Conclusion

The effect of saponin of *Momordica cymbalaria* on den animal shows decreased level of lipid peroxidation and increases the levels of CAT, SOD, GSH, SGPT, SGOT, ALP, total bilirubin and direct bilirubin levels in liver. The results of the present study show tha the saponin of *Momordica cymbalaria* modulating the antioxodant defences both enzymatically and non-enzymatically. the results indicate that saponin of *Momordica cymbalaria* have heptoprotective effect against DEN induced liver tumor.

Hence it was concluded that saponin of *Momordica cymabalria* have hepatoprotective effect.

References

[1] Siegel R, Ma J, Zou Z, et al. Cancer statistics, *CA cancer J Clin*, 2014; 64: 9-29.

[2] Bosch, F.X., Ribes, J. and Borras, J. Epidemiology of primary liver cancer. *Shemin. Liver. Dis*., 1999; 19: 271-285.

[3] Lindhe, B., Porsch-Hallstrom, I., Gustafsson, J.A. and Blanck, A. Effects of neonatal and adult castration and of testoster- one substitution in male rats on growth of enzyme-altered hepatic foci in the resistant hepatocyte model. *Cancer Res*., 1990; 50: 2679-2682.

[4] Nakatani, T., Roy, G., Fujimoto, N., Asahara, T. and Ito, A. Sex hormone dependency of diethylnitrosamine-induced liver tumors in mice and chemoprevention by leuprorelin. *Jpn. J. Cancer Res*., 2001; 92: 249-256.

[5] Kang, J.S., Ahn, B., Kim, C.K., Han, B.S., Che, J.H., Kim, S., Jang, D.D. and Yang, K.H. Suppression of chemically-induced liver tumors by castration or estradiol-3-benzoate treat- ment in F344 rats. *Oncol. Rep*., 2005; 14: 377-382.

[6] Dragan, V.P., Vaughan, J., Jordan, V.C. and Pitot, H.C. Comparison of the effects of tamoxifen and toremifene on liver and kidney tumor promotion in female rats. *Carcinogenesis*, 1995; 16: 2733-2741.

[7] Dragan, Y.P., Xu, Y.D. and Pitot, H.C. Tumor promotion as a target for estrogen/antiestrogen effects in rat hepatocarcino- genesis. *Prev. Med*., 1991; 20: 15-26.

[8] El-Serag, H.B. and Rudolph, K.L. Hepatocellular carci- noma: epidemiology and molecular carcinogenesis. *Gastroenterology*, 2007; 132: 2557-2576.

[9] El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; 132:2557–2576.

[10] McKillop IH, Schrum LW. Role of alcohol in liver carcinogenesis. *Semin Liver Dis* 2009; 29:222–232.

[11] Voigt MD. Alcohol in hepatocellular cancer. *Clin Liver Dis* 2005; 9:151–169.

[12] Brandon-Warner E, Walling TL, Schrum LW, McKillop IH. Chronic ethanol feeding accelerates hepatocellular carcinoma progression in a sexdependent manner in a mouse model of hepatocarcinogenesis. *Alcohol Clin Exp Res* 2012; 36:641–653.

[13] Veerendra KP. Evaluation of antidiabetic activity of *Momordica cymbalaria* and Momordica dioica [dissertation]. Visveswarapura institute of pharmacy: Rajiv Gandhi University of Health Sciences, Bangalore 2007.

[14] Raju K, Balaraman R. Antidiabetic mechanism of saponins of Momordica cymbalaria. *Phcog Mag* 2008; 4(15):197-205.

[15] Raju K, Balaraman R, Hariprasad, Vinoth KM. Cardioprotective effect of *Momordica cymbalaria* Fenzl in rats with isoproterenol-induced myocardial Injury. *Journal of Clinical and Diagnostic Research* 2008; 2:699-705.

[16] Elizabeth A. Evaluation of antifertility activity of roots of *Momordica cymbalaria* Fenzl [dissertation]. Visveswarapura institute of pharmacy: Rajiv Gandhi University of Health Sciences, Bangalore 2005.

[17] Mushirbhai M. Evaluation of antihyperlipidimic activity of saponins fraction of roots of *Momordica cymbalaria* [dissertation]. Visveswarapura institute of pharmacy: Rajiv Gandhi University of Health Sciences, Bangalore 2007.

[18] Koneri R, Balaraman R, Saraswathi CD. Antiovulatory and abortifacient potential of the ethanolic extract of extract of roots *Momordica cymbalaria* Fenzl in rats. *Indian J Pharmacol*. 2006; 38:111-14.

[19] Firdous SM, evaluation of hepatoprotective, analgesic and anti-inflammatory activity of saponins of *Momordica cymbalaria* Fenzl and *Momordica dioica* Roxb [dissertation]. Visveswarapura institute of pharmacy: Rajiv Gandhi University of Health Sciences, Bangalore 2007.

[20] Koneri R, Balaraman R, Firdous, Vinoth K M. Hepatoprotective Effects of *Momordica cymbalaria* Fenzl against carbon tetrachloride Induced hepatic Injury in Rats. *Pharmacologyonline.* 2008; 1:365-374.

[21] Jahn MS,vani G, shyamaladevi CS. Anti-carcinogenic effect of solanum trilobatum in diethyl nitrosamine induced and phenobarbital promoted hepatocaarcinogenesis in rats. *Asian J Biochem* 2011; 6(1):74-81.

[22] Surender RP, Hari KL, Ashok RV, Narshima RY. Chemoprotective effect of *Bombax malabaricum* DC against N-Nitrosodiethylamine induced hepatocellular carcinoma. *Int J Curr pharm Res* 2011, 3(1):93-6.

[23] Fauziah O, Hanchi P, Yogespiriya S, Asmah R, Reducing effect of *strobilanthes crispus* leaf in hepatocarcinogenesis Rats. *Int J cancer Res* 2005; 1(2):109-12.

[24] Natesan S, Badami S, Dongre SH, Godavarthi A, Antitumor activity and antioxidant status of methanol extract of *careya arborea* bark against Dalton's lymphoma ascites-inducces ascetic and solid tumor in mice, *J Pharmacol Sci* 2007;103:12-23.

[25] Xitusan Z, Huilin Z, Shuwei Z, Qiang Y and Lijiang X, Sesquiterpenoids from *Bombax malabaricum, J. Nat. prod* 2007;70(9):1526-1528.

[26] Urtusan RC Chapman JD, Raleigh JA, Franko AJ, and Koch CJ, Binding of 3H- misonidazole to solid human tumors as a measure of tumor hypoxia, *International Journal of Radiation Oncology, Biology, Physics* 1986;12:1623-7.