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Evaluation of Lipid Profile Nature of Solanum Xanthocarpum Leaves in Diethylnitrosamine Induced Hepatocellular Carcinogenesis on Wistar Rats

KEYWORDS: Solanum xanthocarpum,Diethylnitrosamine, Hepatocellular carcinoma, Antioxidant.

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ABSTRACT *Antioxidants are one of the key role players in carcinogenesis, several natural antioxidants were shown to have anticancer effects. In the present investigation the efficacy of Solanum xanthocarpum(SXC) Schrad&Wendl on the antioxidant status of Diethylnitrosamine (DEN) induced liver carcinogenesis in male Wistar albino rats were assessed. The rats were divided into six groups. The rats in the group 1 and 2 were normal control and SXC aqueous leaves extract control respectively. Groups 3, 4, 5 and 6 were administered with DEN (0.01%) in drinking water for 16 weeks to induce hepatocellular carcinoma (HCC). Starting one week prior to DEN administration group 4, 5 and 6 rats were treated with SXC aqueous leaves extract (75, 150 and 300mg/kg bw) day in diet for 16 weeks. After the experimental period of serum glutamic oxaloacetic acid-transaminase (SGOT) and serum glutamic phosphoacetic acid-transaminase (SGPT), alkaline phosphatase (ALP), albumin, bilirubin, total protein, triglycerides (TG), cholesterol (CL) and high density lipoprotein (HDL). In contrast, SXC aqueous leaves extract with DEN treated group 4, 5 and 6 animals showed a significant decrease in the number of nodules with concomitant decrease in the biochemical status. The activities of biochemical analysis liver were improved when compared with hepatocellular carcinoma (HCC) induced group 3 rats. In the present study biochemical analysis were also carried out which supports the chemopreventive action of the SXC aqueous leaves extract against DEN administration during liver cancer progression. These findings suggest that SXC aqueous leaves extract suppresses DEN induced hepatocarcinogenesis by modulating the antioxidant defence status of the rats.*

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for about 90% of all cases of liver cancer and third leading cause of cancer-related death around worldwide (Raphael et al 2012). Approximately 0.5–1 million new cases are diagnosed every year and 595,000 deaths in developing countries (Kalaiselvan et al 2013). In India, HCC is the third leading cause of cancer in men than women (Liu and Wu 2010). A major and etiological risk factor for development of HCC includes hepatitis viral infection, food additives, alcohol, fungal toxins (aflatoxin) and water industerial pollutants (Hussain et al 2012). HCC is a multistep process that involves initial genotoxins insult, clonal expansion from premalignant to malignant lesions and finally resulting tumour progression (Margaret et al 2008).

Diethylnitrosamine (DEN), is a hepatocarcinogen, forms of alkyl DNA adducts, induces chromosomal aberrations and sister chromatid exchanges in liver resulting the development of hepatocarcinogenesis (Perez et al 2010). DEN-induced HCC is the most accepted experimental models and the nitrosamine metabolism in rat liver is similar to human and also with respect to morphology, genomic alterations and gene expression. In DEN-induced HCC, cytochrome isoform of 2E1 produces oxidative stress by generating reactive oxygen species (ROS) (Lin et al 2013). This cellular stress may trigger a series of signalling events associated with progression of benign tumors to malignant neoplasm (Levrero 2006).

India has enormous medicinal plant biodiversity and traditionally used to cure the diseases. In recent years, researchers are focused in the field of phytomedicine to find the drugs from plants (Pant 2009). Plants have the ability for the yield of secondary metabolites like proteins, alkaloids, flavonoids, steroids and phenolic substances which are in turn applied to regenerate wellness and cure many diseases (Merzenuch et al 2010). *Solanum xanthocarpum*(*Solanaceae*), a herb is commonly known as the Indian night shade or Yellow berried night shade (English) (Parmar et al 2010). SXC is known by different name in various different languages in India viz, Kantankattiri (Tamil), Kantkari (Sanskrit), Kateri (Hindi), Bhoringni (Gujarati), Kantkariccunta (Malayalam), Vakudu (Telugu), Nelagulle (Kannad) (Reddy and Rajasekhar Reddy 2014). SXC plant contains alkaloids, sterols, saponins, flavonoids and their glycosides and also carbohydrates, fatty acids, amino acids etc. SXC is a well known medicinal plant in traditional medicinal system (Ayurveda, Siddha, Unani and Folk medicine) (Singh and Singh 2010).

Ayurveda is an ancient form of Indian medicine which deals with plants and parts use as active components for treating diseases. In recent years, there has been considerable prominence on the identification of plant products with anti-oxidant property (Anwikar and Bhitre 2014). SXC has shown anti-inflammatory, anti-oxidant, anti-urolithiatic and hepatoprotective, anti-tumour (Patel et al 2012). There were no studies on chemopreventive efficacy of SXC on HCC. Hence, the present study was designed to evaluate the hepatoprotective effect of SXC against DEN induced liver cancer in experimental rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, weighing 130–150g, procured from the Small Animal Breeding Centre, Muthayammal College of Arts and Science, Rasipuram, Periyar University, Tamil Nadu, India were used. Animals were ac-

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climatized under standard vivarium conditions at 25°C ± 2°C and normal photoperiod (12 h light: dark cycle). The animals were fed with standard rat chow and water *ad libitum*. The food was withdrawn 18–24h before the experiment. The care and use of laboratory animals were done according to the guidelines of the Council Directive CPCSEA, India (Reg. No. 1416/PO/a/11/CPCSEA) about Good Laboratory Practice (GLP) on animal experimentation. All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC).

Chemicals
DEN was purchased from Sigma Lab Ltd, Mumbai, India. All other chemicals and utilized were of analytical grade quality and purchased from HiMedia Laboratories Ltd., Mumbai, India.

Experimental design
The experimental animals were divided into six groups, each group containing six animals, analyzed for a total experimental period of 16 weeks as follows.

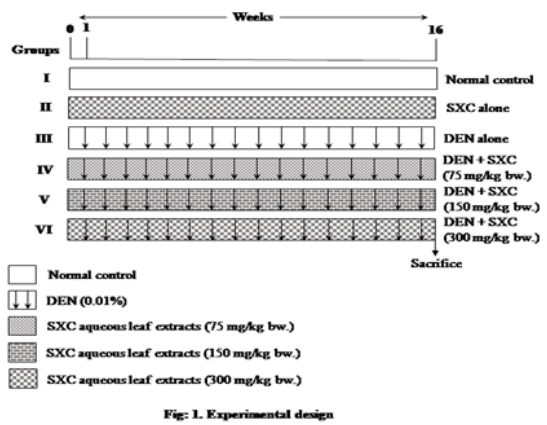

Fig: 1. Experimental design

Group 1: Normal control rats fed with standard pellet diet and pure drinking water.

Group 2: (SXC extracts alone) rats were post orally given SXC extract (300 mg/kg/bw/day) in the form of aqueous suspension daily once a day for 16 weeks.

Group 3-6: rats were induced with DEN (0.01%) alone orally of the experimental period in a week for 16 weeks.

Group 4-6: rats were administered orally given SXC aqueous leaves extracts in a dose dependant manner 75, 150, 300 mg/kg/bw/day., after the last induction of DEN until end of the experimental period 16 weeks respectively.

At the end of 16 weeks, experimental rats (n = 6 per group) were sacrificed. The blood samples were collected from experimental animals.

Source of plant material
The fresh leaves of SXC were collected from Dharmapuri, Tamil Nadu and India. The voucher specimen (Specimen No: CFIS01) was deposited in the Herbarium in Department of Biotechnology, Muthayammal College of Arts and Science. The leaves were collected and washed in running tap water to remove debris, dust particles and then rinsed in distilled water. The leaves were shade dried at normal room temperature for 10–15 days.

Preparation of plant extract
SXC shade dried leaves crushed, into fine powder with mortar, pestle and stored in an air tight container for further use. SXC leaves (300g), were powdered using commercial electrical stainless steel blender and extracted with double distilled water in a soxhlet apparatus (boiling point range 60-80°C) for 8h. The extract was concentrated under reduced pressure of 22-26mm Hg at 45°C, and the residue obtained was stored at 4°C. To prepare the stock solution, one gram of the crude extract was first dissolved in 100mL of double distilled water.

Preparation of serum
Blood samples were collected on the sacrifice day of the experiment from the retro-orbital plexus under light ether anesthesia without any anticoagulant and were allowed to stand for 30 min at room temperature, centrifuged at 2500 rpm for 10 min to separate the serum. The serum obtained was kept at 2–4^{0}C for further use.

Biochemical Estimation
The serum liver marker enzymes in SGPT, SGOT, ALP, albumin, bilirubin, total protein, TG, CL and HDL were performed using a standard kit (photometer 5010, Micro clinical lab Tamil NaduPvt. Ltd).

Statistical analysis
Data are presented as the mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Turkey's multiple comparison method was used to compare the means of different groups by using SPSS 15 student's versions. $P< 0.05$ was considered to be statistically significant in all cases.

RESULTS
DEN control group showed significant reduction in body weight as compared to a normal control group. In SXC aqueous leave extract, the body weight increase when compared to DEN control.

Necrosis and membrane damage release the enzyme into circulation, therefore it can be measured in serum. DEN intoxicated normal Wistar albino rats showed elevated label of ALP, bilirubin and also the total protein level compared to the function of hepatic cell. Increase in the serum level of ALP is due to increase in synthesis and in presence of increasing biliary pressure. Serum total protein, ALP and bilirubin were found to be high level of SGOT and SGPT indicates hepatic damage.

TABLE-1

Groups	Treatment	SGPT (mg/dL)	SGOT (mg/dL)	ALP (mg/dL)	Total Protein (mg/dL)	Albumin (mg/dL)	Bilirubin (mg/dL)
I	Control	51.07 ± 3.89^a	53.34 ± 4.06^a	5.81 ± 0.44^a	7.62 ± 0.58^a	4.02 ± 0.31^a	0.57 ± 0.04^a
II	SXE 300 mg/kg bw	51.08 ± 3.03^a	53.35 ± 3.17^a	5.82 ± 0.39^a	7.63 ± 0.51^a	4.03 ± 0.32^a	0.58 ± 0.03^a
III	DEN (0.01%)	74.89 ± 5.73^b	76.14 ± 5.80^b	10.35 ± 0.79^b	3.64 ± 0.28^b	2.18 ± 0.17^b	1.43 ± 0.11^b
IV	DEN + SXE 75 mg/kg bw	71.03 ± 5.44^c	70.36 ± 5.65^c	8.91 ± 0.68^c	5.13 ± 0.39^c	2.27 ± 0.18^b	0.91 ± 0.07^c
V	DEN + SXE 150 mg/kg bw	65.59 ± 5.02^c	63.18 ± 5.31^c	7.27 ± 0.56^d	6.37 ± 0.49^d	2.73 ± 0.21^c	0.72 ± 0.06^d
VI	DEN + SXE 300 mg/kg bw	50.05 ± 4.73^a	52.20 ± 3.91^a	5.63 ± 0.43^a	7.46 ± 0.57^a	3.88 ± 0.30^a	0.55 ± 0.04^a

Effect of SXC on circulatory liver marker enzymes serum in DEN experimental rats.

Values are expressed as mean ± SD, n=6. In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

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TABLE-2

Groups	Treatment	CL (mg/dL)	TG (mg/dL)	HDL (mg/dL)
I	Control	94.11 ± 7.17^{a}	36.18 ± 2.76^{a}	31.08 ± 2.37^{a}
II	SXE 300 mg/kg bw	94.12 ± 7.19^{a}	36.19 ± 2.73^{a}	32.09 ± 2.35^{a}
III	DEN (0.01%)	35.52 ± 2.71^{b}	95.22 ± 7.25^{b}	10.97 ± 0.84^{b}
IV	DEN + SXE 75 mg/kg bw	52.11 ± 3.99^{c}	76.03 ± 5.82^{c}	15.52 ± 1.19^{c}
V	DEN + SXE 150 mg/kg bw	79.36 ± 6.08^{d}	51.18 ± 3.92^{d}	22.81 ± 1.59^{d}
VI	DEN + SXE 300 mg/kg bw	93.75 ± 7.18^{a}	34.33 ± 2.63^{a}	29.87 ± 2.29^{a}

Effect of SXC on circulatory liver marker enzymes serum in DEN experimental rats.

Values are expressed as mean ± SD, n=6. In each rows, means with different superscript letter differ significantly at $p < 0.05$ (DMRT).

Table: 1 & 2 shows biochemical parameters of serum in control and treated animals. The group III DEN (0.01%) exhibited significant increase in SGPT, SGOT, ALP, albumin, bilirubin, total protein, TG, TC and HDL. The group II (SXC alone 300 mg/kg bw) ($p < 0.05$) exhibited significant decrease in SGPT, SGOT, ALP, Albumin, Bilirubin, CL, TG and significant increase in HDL and Total protein level as compared to vehicle control. The group IV and VI (75, 150, 300 mg/kg bw) exhibited significant decrease in SGPT, SGOT, ALP, albumin, bilirubin, TG and TC level as increase significantly in total protein and HDL level as compared to DEN control. The graphical representation of activity shown by the extract is depicted in table: 1 and 2. The result of this study strongly indicate the anti-carcinogenic effect of SXC aqueous leaves extract against liver injury which may be attributed to its hepatoprotective activity thereby scientifically support its traditional use.

DISCUSSIONS

In Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agent. One of the most versatile plants used in treating HCC is SXC, a member of solanaceae family and it was taken for anti-cancer evaluation in DEN induced Wistar albino rats.

DEN intoxicated normal rat showed elevated label of serum marker enzymes level compared to control group. Serum SGPT, SGOT, ALP, Albumin, Bilirubin, TG, TC, Total protein and HDL are related to the function of hepatic cell. Increase in serum level of ALP is due to increase synthesis in presence of increasing biliary pressure.

Elevated serum levels of SGPT, SGOT, ALP, Albumin, Bilirubin, Total protein, TG, TC and HDL are indicative of poor hepatic function in DEN treated Wistar albino rats. The largest concentration of patients is in Asia and Sub-Saharan countries (Hollstein et al 1993). The development of HCC has also been associated with disorders in plasma lipid and lipoprotein metabolism (Jiang et al 2006). Cancer development is associated with alterations in lipid metabolism, affecting cellular function and growth (Das et al 1998). The development of hepatocyte nodules in rat liver is associated with changes in lipid parameters and oxidative status (Abel et al 2009). Alterations in lipid profiles in malignant tissue are of importance due to their effect on membrane integrity, fluidity and regulation of cellular processes related to growth and cell survival (Bartsch et al 1999:Tapiero et al 2002). The increase in cholesterol level increases the membrane fluidity, regulates membrane permeability and alters internal viscosity and also the internal chemical composition. In the present research it was observed that SXC (300 mg/kg/bw) maintained the lipid profile, hence it can be suggested that SXC (300 mg/kg/bw) may play the role in inhibition of carcinoma progression.

All the observations clearly indicate a chemopreventive function of the extract previous studies conducted by us the extract has anti-tumour activity but no sub acute toxicity (Rajkapoor 2004 and Rajkapoor 2003). Preliminary phytochemical studies have shown the presence of alkaloids and flavonoids in SXC. Flavonoids are known to possess anti-tumourogenic and anti-malignant effects. (Brown 1999).

More over, flavonoids have a chemopreventive role cancer through the induction enzyme affecting carcinogen, metabolism and inhibit various activities of tumour promotors, which is involved in the tumour carcinogenesis (Hertog 1992: Cassady 1990). Chemopreventive effect of SXC aqueous leaves extract may due to the presence of these compounds. Our results clearly indicate a significant chemopreventive effect of SXC aqueous leaves extract. Further studies to characterize the active principles and to elucidate the mechanism action of SXC are in process.

CONCLUSIONS

In the present pharmacological evaluation the aqueous leaves extract of *solanum xanthocarpum*(SXC) belonging to the family solanaceae was extensively investigated for its anticancer activity against DEN induced liver cancer in rats. At the end of our study, a strong conclusion can be drawn that the aqueous leaves extract of SXC possess anticancer activity more or less depending on the dose levels. The aqueous leaves extract at a dose of 300 mg/kg exhibited competent, potent and comparable results. Where as aqueous leaves extract at a dose of 150 mg/kg observed to here moderate level of efficacy promoting SXC plant as a promising anti cancer plant species.

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