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Full Length Research Paper

Biochemical studies on the effect of curcumin and embelin during N-nitrosodiethylamine/phenobarbital induced-hepatocarcinogenesis in wistar rats

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The biochemical effects of administration of embelin a benzoquinone derivative from *Embelia ribes* and curcumin (diferuloyl methane) isolated from *Curcuma longa* against a 2 step hepatocarcinogenic regimen comprising of N-nitrosodiethylamine (DENA)/phenobarbital (PB) was studied in wistar strain male albino rats with respect to lipid profile, renal function tests and levels of blood glucose. Rats administered DENA/PB showed hyper cholesterolaemia, hyper triglyceridaemia, elevated low-density lipoproteins (LDL), free fatty acids (FFA), very-low-density lipoproteins (VLDL) levels and decreased urea levels. Pre- and co-treatment with embelin and curcumin for 14 weeks significantly prevented the biochemical alterations induced by DENA/PB. Results of our study suggest the protective and hypolipidemic effects of embelin and curcumin during chemically- induced hepatocarcinogenesis in wistar rats.

Key words: Embelin, curcumin, N-nitrosodiethylamine, phenobarbital, cholesterolaemia, triglyceridaemia.

INTRODUCTION

Cancer is the major cause of death in the developed, developing and underdeveloped world next only to cardiovascular disease and hence is a major cause of morbidity and mortality worldwide. Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to keep this disease under control (Ravelo et al., 2004). In the recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Modzelewska et al., 2005). One of the oldest, most effective strategies for developing new chemotherapeutics is the isolation and evaluation of chemicals of natural origin. The importance of natural products for drug discovery has been impressive.

Embelin (2, 5 dihydroxy 3-undecyl 1, 4, benzoquinone) is a benzoquinone derivative from *Embelia ribes* Burm. (Family: Myrsinaceae). Embelin is reported to be an effective analgesic (Atal et al., 1984; Zutshi et al., 1990) antifertility agent (Gupta et al., 1989; Agarwal et al., 1986) and is reported to possess significant antiproliferative effects against methylcholanthrene-induced fibrosarcoma in rats (Chitra et al., 1994; Chitra et al., 1995). Curcumin (diferuloyl methane) the active principle of *Curcuma longa* (Family: Zingiberaceae) is documented with several medicinal properties. It is a well known anti cancer agent and is found to induce apoptosis by a p 53 dependent pathway and by activating caspase 3 and 8 (Chan et al., 2000). It is a potent antioxidant (Rukkumani et al., 2003) and anti-inflammatory agent (Handler et al., 2007). Recent studies from our laboratory have confirmed the chemopreventive effects of embelin and curcumin against N-nitrosodiethylamine (DENA)/ phenobarbital (PB)-induced hepatocarcinogenesis in rats (Sreepriya and Bali, 2005).

N-nitrosodiethylamine is a powerful hepatocarcinogen that has been used as an initiating agent in some 2 stage (initiation and promotion) protocols for hepatocarcinogenic studies. It is metabolized to reactive electrophilic reactants that alter the structure of DNA and forms alkyl DNA adducts (Yoshiji et al., 1991).

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Table 1. Levels of blood glucose, blood urea and serum creatinine in the normal and experimental groups of rats.

Group	Urea	Creatinine	Glucose
1	31.49 ± 4.26	1.21± 0.09	73.68 ± 5.01
2	24.92 ± 2.34*	1.2 ± 0.09^{NS}	94.73 ± 6.67***
3	34.87 ± 5.46^{NS}	1.2 ± 0.08^{NS}	75.32 ± 6.74^{NS}
4	30.98 ± 4.29^{NS}	1.2 ± 0.1^{NS}	72.89 ± 5.36^{NS}
5	28.21 ± 2.38*	1.16 ± 0.12^{NS}	73.68 ± 5.68***
6	30.85 ± 4.46*	1.18 ± 0.09^{NS}	80.47 ± 6.21**

Units: mg/ml.
Values are expressed as mean ± SD. (For 6 animals in each group).
Students`t' test - Comparisons are made between group 2, group 3, group 4 Vs group1; group 5, group 6 Vs group 2.
*** P < 0.001; ** P < 0.01; * P < 0.05; NS - non significant.

It induces chromosomal aberrations, micronuclei and sister chromatid exchanges in rat liver (Park et al., 2001). Phenobarbital is an antiepileptic drug that promotes hepa-tocarcinogenesis in rodents when administered subsequent to an initiating carcinogen like N-nitrosodi-ethylamine. Over expression of glutamine synthetase is associated with the beta-catenin mutations in mouse liver tumors during promotion of hepatocarcinogenesis by phenobarbital (Loeppen et al., 2002). The present study was aimed to study the biochemical effects of curcumin and embelin to combat against a 2-step hepatocarcino-genesis induced by DENA (initiator) and PB (promoter) in wistar rats.

MATERIALS AND METHODS

Chemicals

Curcumin, N-nitrosodiethylamine and phenobarbital were procured from sigma chemical company, St Louis, MO, USA. Embelin was kindly isolated and given as a gift by Prof. Narayanan, department of pharmaceutics, Madras medical college, Chennai, India.

Animals

Adult male albino rats of wistar strain weighing between 120 – 140 g obtained from the authentic animal sources of the university were used for the study. They were acclimatized to animal house conditions and were fed on commercial pelleted rat chow (Hindustan Lever Limited, Bangalore, India) and water *ad libitum* (This study was conducted following the ethical guidelines for maintaining animals).

Experimental protocols

The animals were divided into 6 groups of 6 rats each according to the following experimental regimen. Group 1 comprised normal control rats. Group 2 was rats given a single intraperitoneal in-jection of DENA (200 mg/kg). After a brief recovery period of 1 week the rats were given PB (0.05% in drinking water). The admini-stration of phenobarbital was continued for another 13 weeks. Group 3 was rats given curcumin *per se* (100 mg/kg body weight suspended in tween-20 for a period of 14 weeks). Group 4 was rats, which were given embelin *per se* (50 mg/kg body weight suspended in tween-80 for 14 weeks). Group 5 was rats, which were given curcumin 1 week prior to the injection of DENA. The oral administration was then continued throughout the experimental period (14 weeks) along with the administration of phenobarbital. Group 6 were rats, which were given embelin one week prior to the injection of DENA. As in group 5, group 6 rats were orally admini-stered with embelin throughout the experimental period (14 weeks). After the experimental period, the animals were sacrificed by cervical decapitation under ether anesthesia following animal ethics guidelines. Blood and serum obtained from the animals were used for the estimation of glucose (Sasaki et al., 1972), urea (Natelson et al., 1951), creatinine (Broad and Sirota, 1948), cholesterol (Parekh and Jung, 1970), triglycerides (Foster and Dunn, 1973), free fatty acids (FFA) (Hron and Menahan, 1981), very-low-density lipo-proteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

Statistical analysis

Statistical analysis was carried out by student's-t-test. Values are expressed as mean $\pm$ SD and "p" values were determined. Inter group comparisons were made between group 2, group 3 and group 4 Vs group 1; group 5, group 6 Vs group 2.

RESULTS

Table 1 indicates the levels of urea, creatinine and glu-cose in normal and experimental groups of rats. Results indicate that the levels of urea showed a decrease ($P < 0.05$), glucose showed an increase ($P < 0.001$) and there was no significant change in the creatinine levels in the group 2 cancer induced rats; group 3 and group 4 rats given curcumin and embelin *per se* respectively did not show any alteration in the levels of urea, creatinine and glucose as compared to control. Group 5 and group 6 rats showed significant increase in the urea ($P < 0.05$) and decrease in glucose [group 5 ($P < 0.001$), group 6 ($P < 0.01$)] and no significant changes in the creatinine levels as compared to the cancer -induced rats.

Table 2 indicates that levels of cholesterol, triglyce-

Table 2. Levels of cholesterol, triglycerides, free fatty acids, LDL, HDL and VLDL in the normal and experimental groups of rats.

Group	Cholesterol	Triglycerides	FFA	LDL	HDL	VLDL
1	92.30 ± 6.81	52.8 ±4.09	27.31 ± 1.98	49.82 ± 3.97	31.92 ± 2.26	10.56 ± 1.09
2	140.25 ± 9.57***	189.25 ± 9.62***	48.85 ± 3.26***	81.64 ± 7.63***	20.76 ± 1.87***	37.85 ± 2.94***
3	94.89 ± 8.34NS	50.92 ± 4.35 NS	25.81 ± 2.03 NS	52.74 ± 4.39NS	31.97 ± 3.01 NS	10.184 ± 1.12NS
4	90.66 ± 7.34NS	52.16 ± 3.28 NS	26.07 ± 1.98 NS	51.18 ± 5.02 NS	29.05 ± 2.65 NS	10.432 ± 0.97NS
5	116.53 ± 10.62***	106.85 ± 9.12***	36.47 ± 2.77***	70.18 ± 7.09*	24.98 ± 2.05**	21.37 ± 2.05***
6	118.29 ± 12.05***	116.05 ± 9.33***	38.19 ± 3.12***	69.17 ± 7.05**	25.91 ± 2.47**	23.21 ± 1.93***

Unis: mg/dl.
Values are expressed as mean + SD. (For 6 animals in each group).
Students`t' test - Comparisons are made between group 2 and group 1; group 3 and group1; group 4 and group1; group 5 and group 2; group 6 and group 2.
*** P < 0.001; ** P < 0.01; * P < 0.05; NS - non significant.

rides, free fatty acids, LDL, HDL and VLDL in the normal and experimental groups of rats. Group 2 cancer-induced rats showed hypercholesterolemia (P < 0.001), hypertriglyceridemia (P < 0.001), increase in the free fatty acid, LDL, VLDL and decrease in HDL as compared to the control rats. Group 3 and group 4 rats showed no significant change in the above mentioned parameters as compared to control. The group 5 and group 6 rats showed a considerably low levels of cholesterol, triglycerides, free fatty acids, LDL, VLDL and an appreciable increase in the HDL cholesterol as compared to the group 2 rats given DENA/PB.

DISCUSSION

The essential nature of any cancer in humans or in animals continues to challenge many scientists and practitioners interested in the biology, prevention and therapy of this disease. N-nitrosodiethylamine is a powerful heaptocarcinogen known to induce cancer in experimental animals (Pereira et al., 1984). Phenobarbital acts as a tumor promoter when administered subsequent to an initiating carcinogen like N-nitrosodiethylamine. Combination of DENA/PB has been used as a model to screen for the antiproliferative effects of several pharmaceutical agents in experimental animals (Watanabe et al., 2001).

The results of the present study provide substantial support for the protective and hypolipidemic effects of curcumin and embelin during DENA/PB-induced hepatocarcinogenesis in rats. The decrease in urea levels observed in the group 2 rats could be attributed to the declining function of the liver which is the primary site for the synthesis of urea. Several studies earlier, report a decrease in urea levels during liver dysfunction including hepatitis and cirrhosis (Kikuchi et al., 1994; Anandan et al., 1999). During hepatic injury blood urea level declines significantly due to the failure of the liver to convert amino acids and ammonia to urea (Schmike, 1962). Hyperglycemia is usually associated with insulin resistance during gastrointestinal cancers and in the present study also it is possible that the observed increase in glucose levels could be due to the toxicity caused to the pancreatic islet cells and due to the alteration in carbohydrate metabolism by the administration of DENA/PB (Kuklin et al., 2004). The carcinogen exposed rats treated with curcumin and embelin respectively showed an amelioration of the altered biochemical parameters towards normalcy giving an indication of the protective effect of these 2 compounds during DENA/PB-induced hepatocarcinogenesis in rats.

A number of agents that produce liver injury also cause the accumulation of abnormal amounts of fat, predominantly triglycerides, in the parenchymal cells. In general, triglyceride accumulation can be thought of as resulting from an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into the systemic circulation (Plaa and Wewitt 1989). Non esterified fatty acids removed from the circulation or synthesized endogenously are processed thorough 2 major pathways in liver: (a) Mitochondrial beta oxidation for production of metabolic energy and (b) incorporation into complex lipids, especially triglycerides, phosphorlipids, cholesterol esters and glycolipids (Dianzani and Slatter, 1978).

Once synthesized, the complex lipids may be continuously secreted from the liver into the blood. The latter pathway appears to be of greater interest in the triglyceride accumulation observed in the fatty liver caused by the administration of the DENA/PB in our study. Several earlier studies report increased accumulation of serum cholesterol, triglycerides and free fatty acids during DENA/PB induced hepatocarcinogenesis in rats (Jiang et al., 2006). In accordance with these reports we also observed a significant increase in serum cholesterol triglycerides and free fatty acids during DENA/PB-induced hepatic cancer. An appreciable decrease in the aforesaid parameters observed in the group 5 and group 6 rats given DENA/PB + CUR and DENA/PB + EMB respectively gives an indication about the hypolipidaemic effects

of these 2 compounds. This might be indirectly related to the antiperoxidative and inhibitory effects on lipid peroxidation chain reaction, of the 2 compounds during DENA/PB-induced hepatocarcinogenesis in rats (Sreepriya and Bali, 2006).

The decreased levels of HDL and a significant increase in the levels of LDL and VLDL observed in the group 2 cancer-induced rats indicate clearly the hyperlipidaemic conditions caused by exposure to carcinogens. On the contrary, a substantial decrease in the levels of LDL and VLDL coupled with a considerable increase in HDL indicate the beneficial effects of administration of curcumin and embelin during DENA/PB-induced hepatocarcinogenesis in rats. The clear mechanism of action for the observed hypolipidaemic effects is not well understood at this stage of study. It is believed that it could be due to a cumulative protective effect contributed by its antioxidant and antiperoxidative effects coupled with an ability to correct the abnormalities in lipid and lipoprotein metabolism through an increase in the activities of few lipid metabolizing enzymes Viz lecithin cholesterol acyl transferase, lipoprotein lipase and hepatic triglyceride lipase. Further studies are underway in our laboratory to understand the actual mechanism of the drugs contributing to its hypolipidaemic effect.

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