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Research Article

Protective Effects of Embelin and Curcumin Against Diethylnitrosamine / Phenobarbital Induced Experimental Hepatocarcinogenesis in Rats

M.C. Jagadeesh[1]*, M. Sreepriya[1], Geetha Bali [1] & D. Manjulakumari [1]

[1] Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bangalore-560056, India.

Corresponding author

Email:
jagadeeshmbio@gmail.com

Phone No:
+91-9896051979

Fax No:
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ABSTRACT

The effects of administration of Curcumin and Embelin on the levels of certain trace elements and other elements of clinical significance, during experimental hepatocarcinogenesis induced by Diethylnitrosamine/ Phenobarbital (DENA/PB) was studied in Wistar strain male albino rats. The levels of calcium, potassium and sodium were determined in the serum of control and experimental groups of rats. Additionally, the levels of chromium, copper, magnesium, molybdenum and zinc were also determined in the serum, liver and kidney of these rats. Furthermore, lactate dehydrogenase (LDH) activity was also assayed in the serum of these rats. Results revealed both significant and non-significant alterations in the levels of few elements during DENA/PB-induced experimental hepatocarcinogenesis. A statistically significant increase in LDH activity was found in the serum during the cancerous condition. Pre- and co-treatment with Curcumin and Embelin was found to protect the liver against the carcinogenic effects of DENA/PB. This protection was i) due to their ability to prevent changes in the levels of elements studied and ii) by the statistically significant decrease in the activity of LDH that increased in DENA/PB-treated rats and LDH activity in the rats given only Embelin and Curcumin indicating their non-toxic effect. Our present results demonstrate the ability of Embelin and Curcumin to protect against DENA/PB-induced hepatocarcinogenesis in rats.

Key words: Cancer; Curcumin; Diethylnitrosamine; Embelin; Phenobarbital; Rat

INTRODUCTION

Mineral elements serve as structural components of tissues, constituents of the body fluids and vital enzymes in major metabolic pathways essential for proper cell function. Each element has their individual role in the structural and functional integrity of the living cells and organisms. Trace elements play an important role in a number of biological processes by activating or inhibiting enzymatic reactions, by having an effect on the permeability of the cell membranes, maintaining genomic stability, etc. Abnormalities in their metabolism have been demonstrated in many human diseases including cancer. It is therefore believed that these elements would exert actions, direct or indirect on the carcinogenic process and hence have considerable clinical significance. The use of medicinal plants in the treatment of human maladies is known since time immemorial. Many medicinal plants or the active principles isolated from these medicinal plant tissues/organs have been successfully shown to impart protection against an array of diseases and disorders including cancer. Curcumin (diferuloyl methane), is the major yellow pigment isolated from the ground rhizome of the curcuma species, belonging to the Zingiberaceae family. Curcumin has been proved to be a potent anti-oxidant (Kuo et al. 1996), anti-arthritic (Chandra and Gupta 1972), anti-amyloid, anti-inflammatory, anti-microbial and anti-cancer agent (Saeed et al. 2008: Jagadeesh et al. 2009). Embelin is a benzoquinone derivative isolated from dry berries of the plant *Embelia ribes* Burm, which belongs to Myrsinaceae family. In the Indian System of medicine it is popularly known as Vidanga. The main active compound of this plant is embelin (2,5- dihydroxy-3-undecyl-1,4-benzoquinone). Embelin has been reported to be an anti-oxidant (Nazam Ansari et al, 2008), analgesic (Chitra et al, 1994), anticancer (Zutshi et al., 1989) and anti-fertility agent (Radhakrishnan and Alam 1975; Krishnaswamy and Purushothaman 1980). Embelin was found to inhibit

sequentially the TNF-alpha-induced activation of the inhibitory subunit of NF-kB alpha kinase, NF-kB alpha phosphorylation, NF-kB alpha degradation, p 65 phosphorylation and nuclear translocation. Embelin also suppressed the NF-kB-dependent reporter gene transcription induced by TNF-alpha, TNF-receptor-1(TNFR1), TNFR1-associated death domain protein, TNFR-associated factor-2, NF-kB-inducing kinase and IkB alpha kinase but not by p65. Embelin was reported to down-regulate gene products involved in cell survival, proliferation, invasion and metastasis of the tumor (Kwang et al. 2006).

In the present study, a combination of Diethylnitrosamine (DENA) and Phenobarbital (PB) was chosen to induce experimental hepatocarcinogenesis in Wistar strain male albino rats. This carcinogenic model was chosen because nitrosamine is a hepato-specific carcinogen predominantly affecting the liver and the biochemical, morphological and histological changes induced by DENA/PB in rats mimics human hepatocellular carcinoma (Macejova and Britko 2001). Hence this model was chosen to evaluate the anti-cancer effects of embelin and curcumin in the present investigation. The levels of trace elements of clinical significance namely, chromium, copper, molybdenum, nickel, zinc, magnesium, sodium, potassium and calcium were studied during carcinogenic, treated and normal conditions. Moreover, the ability of embelin and curcumin to prevent any major alterations in the levels of these elements, if any, was also studied.

MATERIALS AND METHODS

Chemicals

Curcumin, N-nitrosodiethylamine and Phenobarbital were procured from Sigma Chemical Company, St Louis, MO, USA. Embelin was a kind gift from Dr. Narayanan, Assistant Professor, Department of Pharmaceutical Sciences, Madras Medical College, Chennai 600 003, India.

Animals

Adult male Wistar rats weighing 160-200 g were used for this sudy. They were acclimatized to animal house conditions and were fed with commercial pelleted rat chow (Hindustan Lever Limited, Bangalore, India) and water *ad libitum*. The study on the animals was conducted strictly following the national animal ethics guidelines.

Segregation of the animals and animal experimentation

Animals were divided into six groups of six animals each according to the following experimental regimen. Group 1 comprised normal control rats. Rats of Group 2 were given a single intraperitoneal injection of DENA (200 mg/kg). After one week, the rats were given PB (0.05% in drinking water) for 13 weeks. Animals of Group 3 were given curcumin orally (100 mg/kg/day) in Tween-20, for 14 weeks. Group 4 comprised rats which were given embelin orally (50 mg/kg/day) in Tween-80, for 14 weeks). Animals of Group 5 were given curcumin one 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 treated with embelin (50 mg/kg p.o.) as described for animals of Group 5. Body weight was recorded at the end of every week for 14 weeks. After the experimental period, the animals were killed by cervical decapitation under ether anesthesia following animal ethics guidelines.

Element analysis

Processing of samples for element analysis

Five-hundred mg of accurately weighed tissue or appropriate volume of serum was placed in a conical flask followed by the addition of 2.5 ml of de-ionized water and 1 ml of 1:1 mixture of concentrated nitric acid and perchloric acid. The samples were digested on a water bath till the clear solution was obtained. The digest was filtered through Whatman filter paper and mixed up to 10 ml with de-ionized water by thorough mixing of the solution. The digest was used for the estimation of Ca^{++}, Cr, Cu, Mg^{++}, Mb, K^{+}, Na^{++} and Zn against respective standards. The element analysis was carried out in a GBC 32plus Atomic Absorption Spectrophotometer (GBC equipments Inc., USA).

Lactate dehydrogenase (LDH) was assayed according to the method of King (1965b).

Statistical analysis

Statistical analysis was carried out by Student's *t*-test. Values are expressed as mean ± SD and *P* values were determined. Inter-group comparisons were made. Comparisons were made between Group 2 vs Group 1; Group5, Group 6 vs Group 2; Group 3, Group 4 vs Group 1.

RESULTS AND DISCUSSION

Trace elements play a significant role in controlling several biochemical pathways and many serve as cofactors for several enzymatic reactions. Deficiency or abnormally high levels of these elements may augment several diseased conditions and show clinical manifestations. Minerals are essential elements in many biological functions such as electron transport chain and biological oxidation in mitochondria. Alteration of cell mineral metabolism has been reported to be an important pathogenic step in DENA / PB-induced hepatocarcinogenesis (Thirunavukkarasu and Sakthisekaran 2003).

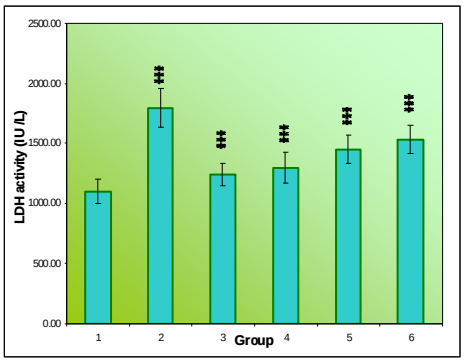
Of the elements studied in current investigation, sodium and potassium play an important role in membrane transport system, whereas magnesium participates in many biochemical reactions which involve a variety of other nutrients, enable muscles to relax, decreases the activity of sodium and potassium pump resulting in a change in membrane potential (Fischer and Giroux 1987). Molybdenum is an inorganic micro mineral, which has a multifaceted role and is a structural component of enzymes *viz* xanthine oxidase, aldehyde oxidase, sulfate oxidase, etc. Molybdenum plays an important role in purine and pyrimidine metabolism and deficiency and can induce growth retardation, whereas excessive unbalanced molybdenum will affect copper absorption (Almli et al. 2005).

Zinc and copper are also essential nutrients. Zinc is required for the synthesis of proteins for muscles, skin, nerves and brain tissue and in protection against infections and disease. Copper on the other hand is required for the synthesis of bones, cartilage, hair, blood and neurotransmitters. It acts both as a pro-oxidant and anti-oxidant (Dorea et al. 1982). Chromium is important to ensure proper control of sugar in the body and helps in the functioning of insulin (Naess, 1992). Alteration of calcium homeostasis and calcium accumulation in liver parenchymal cells has many deleterious effects including modification of cytoskeleton, activation of phospholipases resulting in the perpetuation of cell membrane damage and loss of normal mitochondrial function (Bernardi and Petronilli 1996; John, 1990).

The levels of sodium, potassium, and calcium in the serum of control and experimental groups of rats indicate a non-significant increase in sodium level in the Group 2 cancer-induced rats and non-significant decrease in the Group 3, Group 4, Group 5 and Group 6 rats. The level of potassium showed a non-significant increase in all the groups studied as compared to control animals. The level of calcium showed a non-significant increase in the Group 3, Group 4 and Group 5 rats, whereas there was no change in the levels in the Group 2 and Group 6 rats

Figure 1: Activity of LDH in the serum of Control and Experimental groups of animals

[Values are expressed as mean ± SD. (n=6). Student's *'t'* test - Comparisons are made between Group 2 Vs Group 1, Group 3 & Group 4 Vs Group 1, Group 5 & Group 6 Vs Group 2. *** P<0.001, ** P < 0.01, * P < 0.05, NS- Non significant.]



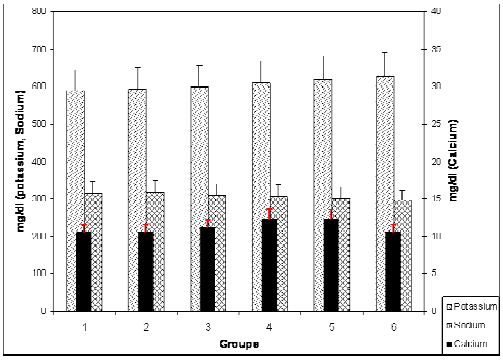


Figure 2: Levels of calcium, potassium and sodium in the serum of control and experimental groups of rats

Values are expressed as mean ± SD. (n=6). Student's *'t'* test - Comparisons are made between Group 2 Vs Group 1, Group 3 & Group 4 Vs Group 1, Group 5 & Group 6 Vs Group 2. *** P<0.001, ** P < 0.01, * P < 0.05, NS- Non significant.

as compared with control (Figure 1).

The levels of magnesium, molybdenum, zinc, copper and chromium in the serum of control and experimental groups of rats are shown in Figures 2 and 3. Results indicate that the level of magnesium was increased (statistically not significant) in the Group 2, Group 3, Group 4, Group 5 and Group 6 rats as compared to control. The level of molybdenum was decreased (statistically non significant) in the Group 2 whereas the Group 3, Group 4 Group 5 and Group 6 rats showed an increase (P<0.05) in the levels over control rats. There were no significant alterations in the levels of zinc and copper in the experimental groups studied as compared with control. Chromium levels registered a decrease in all the experimental groups of rats as compared to control. In particular, the Group 3 and Group 5 rats registered a statistically significant decrease (P<0.001) in the levels of chromium.

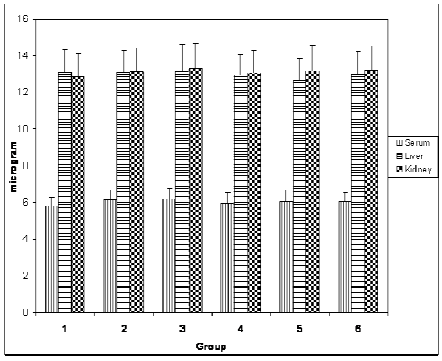
The levels of magnesium, molybdenum, zinc, copper and chromium were estimated in the liver and kidney of control and experimental groups of rats. In liver, the levels magnesium, molybdenum and copper showed non-significant alterations in all the groups studied as compared to control. The Group 2, Group 5 and Group 6 rats showed a statistically significant decrease (P<0.01) in the zinc levels as compared to control whereas the level of chromium was significantly reduced in the Group 2 (P<0.001) and non significant alterations were observed in Group 3, Group 4, Group 5 and Group 6 rats as compared with Group 1 (Figures 2 & 4).

In the kidney, levels of magnesium, molybdenum and copper showed no significant alterations in all the groups studied over control. The level of zinc was significantly increased (P<0.001) in Group 2, Group 5 and Group 6 and non-significant alterations were observed in Group 3 and Group 4 as compared to Group 1. Chromium level showed a significant increase in Group 2, Group 5 and Group 6 (P<0.001), and non-significant alterations in Group 3 and Group 4 as compared with Group 1 (Figures 2 & 5).

The non-significant increase in the levels of magnesium in the serum, liver and kidney in all the groups studied as compared to control in our study shows that there was a mild change in the sodium potassium pump resulting in a change in the membrane potential. This could be due to the changes in the levels of sodium, potassium and calcium which are all inter-related and play a very significant role in

Figure 3: Levels of copper in the serum, liver and kidney of control and experimental groups of rats

[Units: serum: µg / ml. Kidney, Liver: µg / gm. Values are expressed as mean ± SD. (n=6). Student's *'t'* test - Comparisons are made between Group 2 Vs Group 1, Group 3 & Group 4 Vs Group 1, Group 5 & Group 6 Vs Group 2. *** P<0.001, ** P < 0.01, * P < 0.05, NS- Non significant.]



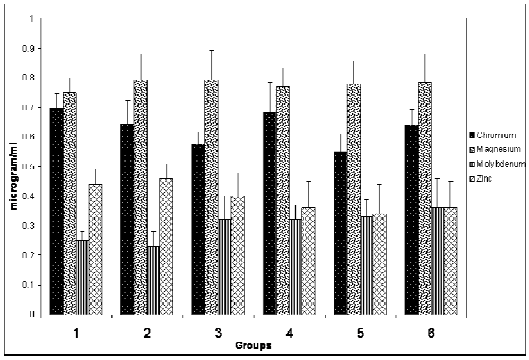


Figure 4: Levels of chromium, magnesium, molybdenum and zinc in the serum of control and experimental Groups of rats.

Values are expressed as mean ± SD. (n=6). Student's *'t'* test - Comparisons are made between Group 2 Vs Group 1, Group 3 & Group 4 Vs Group 1, Group 5 & Group 6 Vs Group 2. *** P<0.001, ** P < 0.01, * P < 0.05, NS- Non significant.

maintaining the membrane potential.

The molybdenum level in the serum showed a non-significant decrease in Group 2 and significant increase in Group 3, Group 4, Group 5 and Group 6 rats (P<0.05). However, no significant alterations in the molybdenum level were found in all the groups studied in both liver and kidney as compared to control. This indicates a mild alteration in purine and pyrimidine metabolism. Zinc and copper levels remained unaltered in the serum. On the contrary, a decrease in the level of zinc in the liver and an increase in its level in the kidney of the Goup2, Group 5 and Group 6 rats as compared with control showed that there could be an imbalance in the utilization and absorption of zinc during cancer conditions. Decreased level of chromium in the liver concomitant with an increase in the kidneys in the Group 2, Group 5 and Group 6 rats indicate alteration in carbohydrate metabolism and impaired excretion of chromium from the body during cancerous conditions.

Of all the macromolecules that leak from the damaged tissues, enzymes are the best markers of tissue damage because of their tissue specificity and catalytic activity. Determination of the activity of hepatic enzymes released into the blood by the damaged liver is one of the most useful tools in the study of hepatotoxicity (Plaa and Wewitt 1989). The clinical and diagnostic values associated with LDH enzyme have long been recognized (Agrawal et al. 1986). Since liver is the main organ that is primarily affected by toxic agents, the examination of serum enzyme activity has been found to be of great importance in the assessment of liver damage. Activity of LDH (Figure 6) was significantly increased (P<0.001) in the Group 2 cancer induced rats, as compared to control rats. In toxic liver injuries, critical changes in LDH activities can be observed in the serum and liver depending on the kind of noxa and extent of cellular damage. Increased activity of this diagnostic marker of hepatic function in Group 2 rats are implicative of the degree of hepatocellular dysfunction caused by the administration of DENA/PB. Increased LDH activity also represents a non-specific alteration in the plasma membrane integrity and/or permeability as a response to DENA/PB challenge. Embelin and Curcumin used in the present study seems to offer significant protection as evident from the decreased activities (P<0.001) of LDH in Group 5 and Group 6 rats as compared to Group 2 rats. This also suggests an ability of the drugs to prevent membrane fragility thereby decreasing the leakage of marker enzyme into circulation via the blood stream. It could also be reasoned that Curcumin and Embelin protect the liver against the carcinogenic effects of DENA/PB. Data in Figure 9 reveals that there is no significant change in the activities of the diagnostic marker of hepatic function in the Group 3 and Group 4 rats given Curcumin *per se* and Embelin *per se*, respectively, as compared to Group 1, thereby showing the absence of any adverse toxic effects of the drug on liver at the dosage employed in the study (Emad, 2007; Nermin et al. 2008). Thus the results of the present study provide strong support to the observation on the protective effects of Embelin from *Embelia ribes* and Curcumin from *Curcuma longa* during DENA/PB-induced hepatocarcinogenesis in Wistar rats.

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REFERENCES

Almli, B.M.; Mwase, T.; Sivertsen, M.M. & Musonda, A. F. 2005. Hepatic and renal concentrations of 10 trace elements in crocodiles (*Crocodylus niloticus*) in the Kafue and Luangwa rivers in Zambia. ***Science of the Total Environment*** 337(3):75-82. http://dx.doi.org/10.1016/j.scitotenv.2004.06.019 PMid:15626380

Agrawal, S.; Chauhan, S. & Mathur, R. 1986. Antifertility effects of embelin in male rats. ***Andrologia*** 18(2):125-31. http://dx.doi.org/10.1111/j.1439-0272.1986.tb01749.x

Bernardi, P. & Petronilli, V. 1996. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. ***Journal of Bioenergetics and Biomembranes*** 28:131-137. http://dx.doi.org/10.1007/BF02110643 PMid:9132411

Chandra, D. & Gupta, S.S. 1972. Anti-inflammatory and anti-arthritic activity of volatile oil of Curcuma longa (Haldi). ***Indian Journal of Medical Research*** 60:138-142. PMid:5029117

Chitra, M.; Sukumar, E.; Suja, V. & Devi, C.S. 1994. Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. ***Chemotherapy*** 40(2):109-13. http://dx.doi.org/10.1159/000239181 PMid:7510605

Dorea, J.G.; Ferraz, E. & Queiroz, E.F. 1982. Effects of anovulatory steroids on serum levels of zinc and copper. ***Archivos latinoamericanos de nutrición*** 32:101-110. PMid:7181622

Emad, M.E.Z. 2007. Isoenzyme Pattern and Activity in Oxidative Stress-Induced Hepatocarcinogenesis:The Protective Role of Selenium and Vitamin E. ***Research Journal of Medicine and Medical Sciences*** 2(2):62-71.

Fischer, P.W. & Giroux, A. 1987. Effects of dietary magnesium on sodium-potassium pump action in the heart of rats. ***Journal of Nutrition***117:2091-2095. PMid:2826728

Jagadeesh, M.C.; Sreepriya, M.; Geetha, B. & Manjulakumari, D. 2009. Biochemical studies on the effect of curcumin and embelin during N-nitrosodiethylamine/ Phenobarbital induced-hepatocarcinogenesis in wistar rats. ***African Journal of Biotechnology*** 8(18):4618-4622.

John, L.F. 1990. The Role of Calcium ions in toxic cell injury. ***Environmental Health Perspectives*** 81:107-111.

Plaa, G.L.; Wewitt, W.R. & Hayes, W.A. 1989. Principles and methods of toxicology, 2nd edn. Raven Press Ltd. pp. 599.

Kuo, M.L.; Huang, T.S. & Lin, J.K. 1996. Curcumin, an antioxidant and anti-tumor promoter, induces apoptosis in human leukemia cells. ***Biochimica et Biophysica Acta*** 1317(2):95-100. PMid:8950193

King, J. 1965. In: Practical Clinical Enzymology. Van D Nostrand Company, pp. 363.

Krishnaswamy, M. & Purushothaman, K.K. 1980. Antifertility Properties of Embelia ribes: (Embelin). ***Indian Journal of Experimental Biology*** 18:1359-1360. PMid:7216303

Kwang, S.A.; Gautam, S. & Aggarwal, B.B. 2006. Embelin, an Inhibitor of XIAP, Blocks Nuclear Factor-B (NF-B) Signaling Pathway Leading to Suppression of NF-B regulated Anti-apoptotic and Metastatic Gene Products. ***Molecular Pharmacology Fast Forward*** 28787:1-38

Macejova, D. & Britko, J. 2001. Chemically induced carcinogenesis: A comparison of 1-methyl-1-nitrosourea, 7,12-dimethyl benzanthracene, diethylnitroso-amine and azoxymethan models. ***Endocrine Regulations*** 35:53-39. PMid:11308997

Nazam Ansari, M.; Bhandari, U.; Islam, F. & Tripathi, C.D. 2008. Evaluation of antioxidant and neuroprotective effect of ethanolic extract of Embelia ribes Burm in focal cerebral ischemia/reperfusion-induced oxidative stress in rats. ***Fundamental & Clinical Pharmacology*** 22(3):305–314. http://dx.doi.org/10.1111/j.1472-8206.2008.00580.x

Nermin, A.H.S.; Shohda, F. & Manal, I. 2008. Diethylnitrosamine-induced hepatocarcino- genesis in rats: possible chemoprevention by blueberries. ***African Journal of Biochemistry Research*** 2(3):81-87.

Naess, K. 1992. The significance of chromium for metabolic cardiovascular syndrome. ***Tidsskrift for Den norske legeforening*** 112(20):2672-2673. PMid:1412297

Radhakrishnan, N. & Alam, M. 1975. Antifertility activities of Embelin in albino rats. ***Indian Journal of Experimental Biology*** 13:70-71. PMid:1158401

Tajbakhsh, S.; Mohammadi, K.; Deilami, I.; Zandi, K.; Fouladvand, M.; Ramedani, E. & Asyesh, G. 2008. Antibacterial activity of indium curcumin and diacetylcurcumin. ***African Journal of Biotechnology*** 7(21):3832-3835.

Thirunavukkarasu, C. & Sakthisekaran, D. 2003. Effect of dietary selenite on N-nitrosodiethylamine-induced and phenobarbital promoted multistage hepatocarcinogenesis in rat: reflection in some minerals. ***Biomedicine & Pharmacotherapy*** 57(9):416-421. http://dx.doi.org/10.1016/j.biopha.2003.08.023

Zutshi, U.; Johri, R.K.; Atal, C.K. 1989. Possible interaction of potassium embelate, a putative analgesic agent, with opiate receptors. ***Indian Journal of Experimental Biology*** 27(7):656. PMid:2561116