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Influence of aqueous extract of *Cinnamomum zeylanicum* on the progression of cancer in diethylnitrosamine induced rat liver

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ABSTRACT

Cinnamon (Cinnamon zeylancium) has been used to investigate its effect on anti-oxidant status, alpha feto protein, serum transaminases and histology of Liver in Diethylnitrosamine induced rats. Wistar strain albino rats weighing 130-250 grams weight, were divided into five groups of five animals each. A low dose of 0.5 mg/100g BW/day and high dose of 1.0 mg/100g BW/day twice a week as intraperitoneal injection was fixed for DEN, while cinnamon was supplemented at a dosage of 0.2ml/100g BW/day daily orally. Group I served as normal control and received normal saline 1ml/100gm BW/day for 45 days. Group II given low dose DEN for 45 days. Group III given high dose DEN for 45 days. Group IV given low dose DEN + Cinnamon supplementation, while Group V given high dose DEN + Cinnamon supplementation. At the end of the experimental period, all the animals were sacrificed, blood collected and processed for the analysis of anti-oxidant enzymes, alpha feto protein and serum transaminases. Body is opened and Liver was removed and assessed for histopathology. A significant increase in the activity of SOD, CAT, GSH, GPX, and GST have been observed in DEN treatment groups. Likewise, cinnamon supplementation is seen to bring about an overall slight increase in the activities of SOD, CAT, GSH and GPX. The level of TBARS was significantly increased in DEN treated as well as cinnamon supplemented groups. The level of AFP was seen to be significantly increased in both the DEN treated groups and supplementation with cinnamon increased the level of AFP in the low dose while a significant increase can be seen in the high dose supplementation group. A significant increase in the activities of transaminases in both the DEN treated groups was observed. Cinnamon supplementation Treatment with high dose of DEN was observed to increase the activity of ALP, with a decrease in the low dose DEN group. Supplementation with cinnamon seems to increase the ALP activity in both the dosages in a dose dependant way. Histological results showed the presence of large tumour cells in trabecular formation, atypic nuclei showing high mitotic activity and severe hepatocyte necrosis in liver. Supplementation with cinnamon improves the histoarchitecture of hepatic cells with slight regeneration of tubular structure, restoration of nuclear shape with presence of dense glycogen granules.

Key words: Cinnamon, Diethylnitrosamine, Anti-oxidant enzymes, Transaminases, Liver Histology, TBARS and AFP.

INTRODUCTION

Herbal and natural products represent one of the most common forms of complementary and alternative medicines. Medicinal plants have played an important role in the pharmacology and medicine for many years. It is estimated that about 80 percent of the world population relies on botanical preparations as medicine to meet their health needs. Cancer, known medically as a malignant neoplasm, is a broad group of varied diseases, all involving cell growth. Every year atleast 20,000 people die worldwide from cancer. The liver is the second largest organ in the human body after skin, and is the largest internal organ. Liver cancer is the second most common cancers in the world and

most common in Asia, Africa and Southern Europe. Liver cancer or Hepatic cancer (from the greek hepar, meaning Liver) is a cancer that originates in the liver, and are malignant tumors that grow on the surface or inside the liver. Diethylnitrosamine (DEN) is a hepatocarcinogen and is normally used as a carcinogen to induce live cancer in animal models [1]. Several naturally occurring dietary and non-dietary phytochemicals have shown enormous potential in the prevention and treatment of several cancers, especially those of the gastrointestinal tract. Cinnamon is a common spice employed in cooking as condiment and flavouring material. As it is said to have anti- clotting, anti-diabetic and anti-oxidant effect, as well as reduce the risk of colon cancer, the extract of cinnamon has thus been employed in the present investigation to study its efficacy on alleviating the production and progression of cancer in liver of diethylnitrosamine induced rats.

MATERIALS AND METHODS

2.1. Selection of the Animal Model

Albino rats, which had comparable absorption, tissue absorption, metabolism and excretion of test compound comparable to that of human beings, were selected for the present study. Wistar strain albino rats weighing about 130-250 grams were selected. The rats were procured and acclimatized to our laboratory conditions for two weeks. The animals were housed in a well ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness. They were fed with standard diet and drinking water was made available *ad libitum.*

Experiments were complied with the ruling of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), New Delhi, India. Registration No. 722/02a CPCSEA dt. 14.12.2006 and the study were permitted by the Institutional Ethical Committee (IEC) of the Bharathiar University.

2.2. Preparation of Cinnamon Extract

15g of cinnamon powder was dissolved in 150 ml of distilled water and placed in a water bath, which was maintained at 60ºC for 2 hours. Then the extract was filtered and stored for experimentation.

2.3. Diethylnitrosoamine (DEN)

Diethylnitrosoamine was purchased from Sigma chemical co. (Bangalore). DEN was dissolved in saline and injected twice a week as intra peritoneal injection at low dose of 0.5 mg/100g BW/day and high dose of 1.0 mg/100g BW/day for 45 days to initiate hepatic carcinogenesis.

2.4. Experimental design

Healthy male albino rats were divided into 6 groups of 5 animals and received the following regimen of treatments.

Group I - Animals received normal saline 1ml/100gm BW/day for 45 days and used as control.
Group II - Animals were injected DEN 0.5mg/100gm BW/day twice a week for 45 days intraperitoneally.
Group III - Animals were injected DEN 1mg/100gm BW/day twice a week for 45 days intraperitoneally.
Group IV - Animals were injected DEN 0.5mg/100g BW/day twice a week intraperitoneally and cinnamon extract given orally 0.2ml/100g BW/day daily for 45 days.
Group V - Animals were injected DEN 1mg/100g BW/day twice a week intraperitoneally and cinnamon extract given orally 0.2ml/100g BW/day daily for 45 days.
Group VI – Animals injected high dose of DEN and cinnamon at a dose of 0.5ml/100g BW/day daily for 30 days, after giving high dose DEN for 45 days.

All the treatments were given between 9:30 to 10:00 hours in the morning. . At the end of the experimental period, all the animals were sacrificed; blood collected and processed for the analysis of anti-oxidant enzymes, alpha feto protein and serum transaminases. Body is opened and Liver was removed and assessed for histopathology.

2.5. ASSAY OF ENZYMIC ANTIOXIDANTS

- Superoxide Dismutase **(SOD)** activity was assayed by the method of Kakkar *et al.* (1984) [2].
- Catalase **(CAT)** activity was assayed by the method of Sinha (1972) [3].
- Glutathione S- transferase **(GST)** was assayed by the method of Habig *et al*. (1974) [4].
- Glutathione peroxidase **(GPx)** was assayed by the method of Rotruck *et al*. (1973) [5].
- Reduced glutathione **(GSH)** was estimated by the method of Ellman (1959) [6].
- Thiobarbituric acid reactive substances **(TBARS)** was estimated by the method of Yagi (1978) [7].
- Alpha feto protein **(AFP)** was estimated by the method of Hirai (1982) [8].

2.6. ESTIMATION OF SERUM TRANSAMINASES

❖ Serum glutamate oxaloacetate transaminase **(SGOT)** and Serum glutamate pyruvate transaminase **(SGPT)** were estimated by the method of Reitman and Frankel (1957) [9].

2.7. ESTIMATION OF ALKALINE PHOSPHATASE

❖ Alkaline phosphatase is determined by colorimetric method.

2.8. HISTOLOGICAL STUDIES

The time- honoured paraffin embedding and haematoxylin- eosin staining technique was used for the preparation of sections. The prepared slides were then analyzed for structural modifications induced by cancer progression.

2.9. STATISTICAL ANALYSIS

Results obtained were tabulated. Statistical analysis was carried out using Dunnetts "t" test. Any significant variation between the control and treated groups were recorded.

RESULTS AND DISCUSSION

3.1.Effect on Body Weight (Table 1)

Table 1 : The effect of Cinnamon extract on Body Weight of Diethylnitrosamine induced male albino rats.

GROUPS	BODY WEIGHT (GRAMS)	
	INITIAL WEIGHT	FINAL WEIGHT
C	142±5.386	189± 7.969
DEN 1	181±2.915	231± 6.783
DEN 2	193±8.000	241± 11.336
DEN 1+ C	154±1.871	210± 3.536
DEN 2+ C	174±9.274	237± 5.386

Values are expressed as Mean ± S.E.M of five rats.
**Significance at 5% level, [a]Significance at 5% level of G2 Vs G4, [b]Significance at 5% level of G3 Vs G5.*
C – Control, DEN 1 - Diethylnitrosamine (Low dose), DEN 2 - Diethylnitrosamine (High dose), DEN 1 + C - Diethylnitrosamine (Low dose) + Cinnamon, DEN 2 + C - Diethylnitrosamine (High dose) + Cinnamon.

No significant changes were seen in the bodyweight of cinnamon supplemented groups on comparison with control. A slight decrease can be observed in the DEN alone treated groups in a dose dependent manner. Orally administrated aqueous suspension of spices like *Allium cepa, Allium sativum, Capsicum annum, Carum carvi* and *Amethum graveolens* caused no significant difference in body weight and organ weight on chronic treatment [10].

3.2. Effect on Liver Weight (Table 2)

Table 2: The effect of Cinnamon extract on Liver Weight of Diethylnitrosamine induced male albino rats.

GROUPS	LIVER WEIGHT (GRAMS)
C	5.802 ± 0.342
DEN 1	7.28 ± 0.473*
DEN 2	7.888 ± 0.552*
DEN 1 + C	7.296 ± 0.143*
DEN 2 + C	5.662 ± 0.133[b]

Values are expressed as Mean ± S.E.M of five rats. Significance at 5% level, [a]Significance at 5% level of G2 Vs G4, [b]Significance at 5% level of G3 Vs G5.*

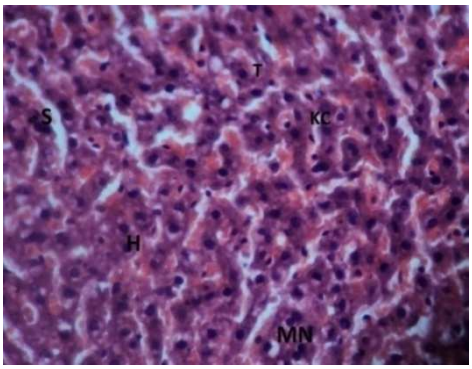
A significant increase can be observed in the weight of liver in all the treatment groups except for the cinnamon treated high dose DEN group. Here, a significant near normal reduction in liver weight is seen on comparison with high dose DEN treatment groups.

Cinnamomum zeylancium treatment caused a reduction in liver weight [11]. Garlic oil (GO) administration significantly inhibited the increase of the nodule incidence and average nodule number per nodule-bearing liver induced by NDEA, improved hepatocellular architecture, and dramatically inhibited NEDA induced elevation of serum biochemical indices in a dose dependent manner [12].

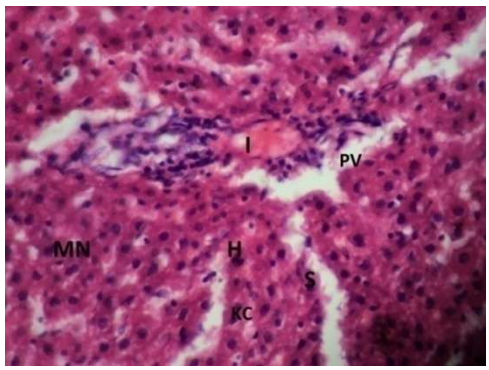
3.3. EFFECT ON LIVER HISTOLOGY (Plate 1)

PLATE 1

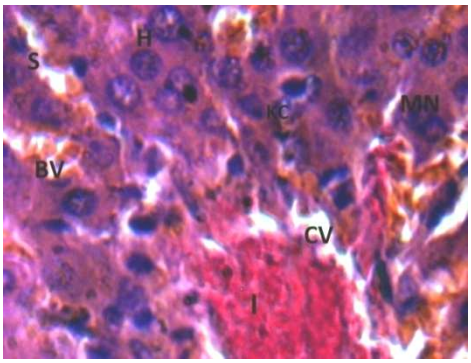
CONTROL LIVER



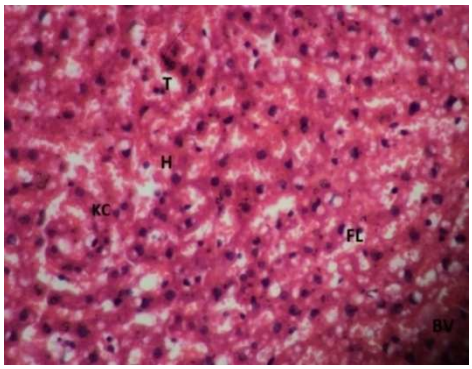
DEN 1



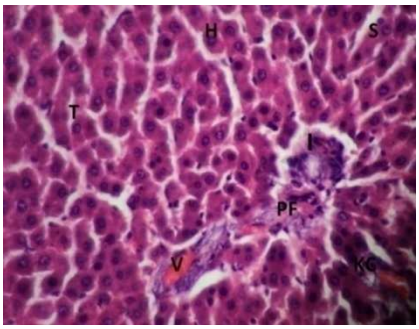
DEN 2



DEN 1 + CINNAMON



DEN 2 + CINNAMON



KC - Kupffer cells, H - Hepatocytes, T - Trabeculae, S - Sinusoids, MN - Mitotic Nucleus, FL- Fatty Lobules, PV- Portal vein, BV - Blood Vessel, I - Infiltration, PV - Perivenular Fibrosis, CV - Central Vein, HVC - Hepatic Vein Congestion.

The control liver consists of a vast interanastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids. The hepatocytes along with vascular channels form organized micro structures with serve as structural and functional units. Innumerable lobules consisting of central vein surrounded by radiating hepatocytes also observed. Portal tracts are triangular to round structures which contain portal veins, terminal branches of hepatic artery and bile ducts embedded in fibrous connective tissue.

The liver treated with low dose of Diethylnitrosamine (DEN) showed the primary appearance of tumor as multifocal, solitary or confluent growths. They were composed of small rounded or polygonal cells arranged occasionally in cords and trabecular formations. High dose of Diethylnitosamine (DEN) seems to have increased the severity of tumor cells. Solid masses of cells separated by blood filled sinusoid like spaces were observed. The tumor cells demonstrate a degree of anisocytosis and pleumorphism. Their cytoplasm was basophilic. Atypic nuclei showing mitotic activity at varying degrees also seen. Intranuclear and intracytoplasmic inclusion bodies also observed. Perivenular inflammatory infiltration with diffuse mild hepatocellular vacuolations also observed, thus expressing a severe hepatic inflammatory reaction.

Supplementation with cinnamon improves the histoarchitecture of hepatic cells with slight regeneration of tubular structure, restoration of nuclear shape with presence of dense glycogen granules. Increase in Kupffer and sinusoidal endothelial cells in hepatic lobules also seen.

Garlic oil (GO) administration significantly inhibited the increase of the nodule incidence and average nodule number per nodule-bearing liver induced by NDEA, improved hepatocellular architecture, and dramatically inhibited NEDA induced elevation of serum biochemical indices in a dose dependent manner [13].

3.4. Effect on Antioxidant Status (Table 3)

Table 3: The effect of Cinnamon extract on Diethylnitrosamine induced alterations in Antioxidant Status

PARAMETERS	C	DEN 1	DEN 2	DEN 1 + C	DEN 2 + C
SOD(U/mg)	2.484±0.184	1.35±0.146*	1.41±0.187*	1.992±0.064*[a]	1.672±0.128*
GPX(U/mg)	5.542±0.230	3.438±0.917*	3.266±0.419*	3.32±0.156*	3.866±0.196*[b]
GST(U/mg)	2.234±0.120	1.022±0.041*	1.138±0.075*	1.22±0.083*	1.02±0.062*
CAT(U/mg)	35.55±0.324	25.35±0.668*	27.78±0.347*	29.704±0.237*[a]	30.9±0.469*[b]
GSH(mg/g)	42.898±0.384	32.354±0.264*	29.614±0.352*	33.672±0.431*[a]	35.312±0.263*[b]
TBARS	1.016±0.069	2.106±0.085*	2.812±0.083*	2.198±0.150*	2.0580.094*[b]
AFP	1.562±0.178	4.152±0.075*	5.214±0.084*	5.048±0.148*[a]	3.074±0.102*[b]

Values are expressed as Mean ± S.E.M of five rats.
**Significance at 5% level, [a]Significance at 5% level of G2 Vs G4, [b]Significance at 5% level of G3 Vs G5.*

On comparison with control, a significant reduction in the activity of superoxide dismutase (SOD) can be observed in the Diethylnitrosamine (DEN) treated groups Supplementation with cinnamon brought about a slight increase in the activity of SOD on comparison with DEN treated groups.

A similar significant decrease in the activities of catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) have been observed in all the DEN treatment groups. Likewise cinnamon supplementation is seen to bring about an overall slight increase in the activities of SOD, CAT and GSH when compared to the DEN treated groups.

The levels of thiobarbituric acid reactive substances (TBARS) was significantly increased in the DEN treated as well as cinnamon supplemented groups.

Garlic oil (GO) administration significantly inhibited NEDA-induced elevation of serum biochemical indices and the mechanistic studies demonstrated that GO counteracted NDEA-induced oxidative stress in rats illustrated by the restoration of glutathione, glutathione-S-transferase levels, and the reduction of the malondialdehyde levels in liver [13]. The aqueous and alcoholic extract of Seabuckthorn (Hippophae rhamnoides) fruit powder treated animals produced significant increase in the levels of total protein, GPx, GST, GRD, SOD and CAT in animals challenged with carbon tetrachloride (CCL4) [14].

3.5. EFFECT ON ALPHA FETO PROTEIN (AFP) (Table 3)

The level of Alpha feto protein (AFP) was seen to be very significantly increased in both the DEN treated groups in a dose dependant manner. Supplementation with cinnamon increase the level of AFP in the low dose group while a significant decrease can be seen in the high dose supplementation group.

Significant increase in the serum levels of AFP in DEN treated group and significant decrease of AFP levels on blue berry supplementation have been observed [15].

3.6. EFFECT ON TRANSAMINASES (Table 4)

The activities of transaminases were significantly increased in both the Diethylnitrosamine (DEN) treated groups. Cinnamon supplementation can be observed to significantly decrease the activity of serum glutamate oxalate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) in both the DEN treated groups.

Treatment with high dose of DEN was observed to increase the activity of alkaline phosphatase (ALP) while a decrease in the activity can be observed in the DEN low dose group. Supplementation with cinnamon seems to increase ALP activity in both the dosages in a dose dependant way.

Table 4 : The effect of Cinnamon extract on Diethylnitrosamine induced alterations in Serum Transaminases and Alkaline Phosphatase

PARAMETERS	C	DEN 1	DEN 2	DEN 1 + C	DEN 2 + C
SGOT(g/dl)	274.06±1.242	1451.64±11.80*	1649.06±29.70*	1423.54±1.886*[a]	1025.24±2.548*[b]
SGPT(g/dl)	194.12±1.708	2150.3±1.503*	1434.56±6.720*	1853.88±2.073*[a]	204.24±0.611*[b]
ALP(U/l)	226.22±1.486	185.14±1.214*	299.66±1.019*	235.66±1.321*[a]	315.36±1.633*[b]

Values are expressed as Mean ± S.E.M of five rats.
**Significance at 5% level, [a]Significance at 5% level of G2 Vs G4, [b]Significance at 5% level of G3 Vs G5.*

The aqueous and alcoholic extract of Seabuckthom *(Hippophae rhamnoides*) fruit powder at dose levels of 400 mg/Kg body weight administered orally for seven days to the carbon tetra chloride (CCl4) challenged rats produced significant reversal of biochemical changes in liver and serum preventing wide range of tissue injury in CCl4 challenged rats as evidenced by significant reduction in GOT, GPT, ALP [14].

CONCLUSION

Through centuries of clinical practices in herbal medicine, a number of candidate drugs have been derived from the herbs or herbal composite formulae for chemoprevention and chemotherapeutic strategy against HCC. Among the herbal toxic effects, hepatotoxicity is the most frequent. The investigation of compounds and composite formula regarding safety and toxicity is needed before definitive clinical guidelines can be made. Therefore cinnamon - an everyday spice has been used in the present study. The antitumor activity of water soluble cinnamon extract has already been tested using various types of cell lines including lymphoma, melanoma, cervix cancer and colorectal cancer in mouse. Its antitumor activity has also been linked with their enhanced pro-apoptotic activity by inhibiting the activities of NF_kB and AP_1 in mouse melanoma model, through target genes such as BCl_2 and BCl-xl. Thus, the potent antitumoral effects of cinnamon extract seen to be mediated by multiple action mechanisms. The present investigation undertook to study the effect of cinnamon extract on the DEN induced changes in liver function based on antioxidant profile, activities of transaminases as well as histopathological exposure. The carcinogenic induction of DEN seems to be effectively counteracted by cinnamon supplementation. In correlation with our present study, [16] have also recorded the highest *in vivo* and *in vitro* anticarcinogenic effect on comparatively evaluating the cytotoxic potential of cabbage, cauliflower and carrot with cinnamon against human $HepG_2$ cancer cell lines. Even though the antioxidant property of cinnamon has been expressed clearly along with its carcinogenesis modulating ability, properties of the active constituents, including their metabolism and toxicity need to be tested, to properly establish cinnamon as a potent chemopreventive agent. Through the present investigation, it can be summarized that cinnamon can be used as a potent chemopreventive antitumour agent, provided, the properties of its active constituents, their metabolism and toxicity, has been assessed at various doses and durations.

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