

Hepatoprotective Activity of Turmeric and Garlic against 7-12, Dimethylbenzanthracene Induced Liver Damage in Wistar Albino Rats

Harmeet Singh¹, P. S. Bedi^{2*} and B. Singh³

¹Department of Biotechnology, SBSPGI, Dehradun, Uttarakhand, India.

²Department of Chemistry, M. M. University, Solan, H. P., India.

³Department of Biochemistry, SBSPGI, Dehradun, Uttarakhand, India.

Research Article

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ABSTRACT

Aim: In present study the antioxidant activity of turmeric was studied in Wistar albino rats.

Study design: The experimental animals were divided in to five groups each containing six animals. Group I served as normal control. All the other four groups, viz. II, III, IV and V were first challenged with 7-12 Dimethylbenzanthracene (DMBA). Thereafter, group III, IV and V received Indole-3-Carbinol, turmeric and turmeric with garlic respectively for four weeks. Group II have Received no intervention other than DMBA.

Methodology: At the end of the study all the animals were sacrificed and the effects of DMBA, Indole-3-Carbinol, Turmeric and Turmeric with garlic were monitored by growth rate during study period and also by assaying the levels of lipid peroxidation (MDA), superoxidisedismutase (SOD), and catalase (CAT) in liver and kidney homogenates. In addition serum alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and Creatinine levels were also studied.

Results: The results showed that the difference in total body weight gain was not significant among all the groups. Body weight in group II was found to be reduced than the body weight on zero day. There was significant decrease in SOD and CAT, but significant increase in MDA in both the tissue homogenates. The levels of AST, ALT, ALP, Creatinine and Urea were significantly increased in group II. Reversal effects of DMBA were shown by group IV and V but they were lower than group III. Cyst formation in liver was observed in group II rats only. Enlargement and paleness of liver was maximum in group II as compared to other treated groups.

Conclusion: In present study the turmeric have shown the reversal effects of DMBA induced carcinogenicity.

Keywords: Carcinogenic DMBA; hepatotoxicity; turmeric; garlic; SOD; CAT; MDA; ALT; AST; ALP; creatinine; urea.

1. INTRODUCTION

Cancer is a class of diseases in which a group of cells display the traits of uncontrolled growth invasion and sometimes metastasis. The cancerous tumour resembles the shape of crab, giving the disease its name cancer. Presently cancer is among biggest health problem worldwide. World Cancer report issued by International agency for research on cancer (IARC) reported in 2003 that cancer rate is set to increase at an alarming rate globally.

Cancer results from a multistage carcinogenesis process that involves three distinguishable but closely connected stages: initiation (normal cell → transformed or initiated cell), promotion (initiated cell → pre neoplastic cell) and progression (pre neoplastic cell → neoplastic cell) Brennan (1975). Among various types of cancer, liver cancer is one of the most frequent occurring cancer that kills more than six lac people around the world annually. Liver cancer is characterised by the presence of malignant hepatic tumour or growth on or in the liver. The most frequent malignant primary liver cancer is hepatocellular carcinoma. Herbs have been used to treat cancer since ancient times, more than three thousand species of herbs used in treating cancer are known in biomedicines. American cancer society defines complementary and alternative medicines simply as anything which is not conventional (Zollman and Vickers, 1999).

DMBA a polycyclic aromatic hydrocarbon, has been used extensively as a model carcinogen in cancer research. DMBA elicits immunotoxicity in the spleen, thymus, liver and bone marrow. It has been shown to suppress both humoral cell mediated immune response in spleen and cultured splenocytes (Ward et al., 1984; Dean et al., 1986; Thurmond et al., 1998). Turmeric (*Curcuma longa*) also called Indian saffron since ancient times is widely cultivated in India, Sri lanka, Belgium, Indonesia and France. Only India accounts for more than 90% of total production of the world. Hence the turmeric powder is one of the most common food flavouring and colouring agent in Asian cuisines. Curcumin is the most active constituent present in turmeric and exert potent biological effects in-vitro and in-vivo (Wealth of India, 1995; Sharma et al., 2005; Agarwal et al., 2007). Many medicinal uses are rendered for the plant especially in India, China and Indonesia, where it is used as house hold remedy as anti inflammatory, antiseptic and dye stuff for silk and wool. The beneficial antioxidant, anti inflammatory and anti tumorigenic effects of Curcumin and garlic in relation to cancer and other chronic diseases are reported (Maheshwari et al., 2006; Karina et al., 2007 ; Shishodia et al., 2007; Sabry et al., 2009; Zakaria, et al., 2010; Canogullari et al., 2010). The present study has been taken up, keeping in view the severity of cancer as a disease yet to be cured.

2. MATERIALS AND METHODS

The experiment was performed on Wistar albino rats obtained from SBSPGI, Dehradun, (UK). A total 30 animals were equally divided in to five groups (N= 06 in each group). The rats were housed in clean polypropylene cages and fed *ad libitum* with commercially available feed and water.

Turmeric rhizomes (*Curcuma longa*) were procured from local market and dried aerielly. Dried rhizomes were ground to powder and this powder was stored in reagent bottle for further use. The details of the groups and treatments are given in (Table 1).

Table 1: Distribution of rats and details of the treatment given

Sl. No.	Group	No. of animals in group (N)	Type	Treatment
1	I	6	Normal Control	NIL
2	II	6	Negative Control	DMBA + TPA + Dexamethazone
3	III	6	Positive Control	DMBA + TPA + Dexamethazone + Indole-3-Carbinol
4	IV	6	Test I	DMBA + TPA + Dexamethazone +Turmeric @ 300mg/Kg body weight
5	V	6	Test II	DMBA + TPA + Dexamethazone +Turmeric @ 150mg/Kg body weight + Garlic 150mg/Kg body weight

Cancer was induced in all the four groups (II, III, IV and V) by administration of DMBA @ 300µg/Kg. Body weight subcutaneous and 100µg topical, thrice a week, dexamethazone @ 1.2 mg per rat twice a week and TPA @ 5µg three doses twice a week. The rats of group three were given an anti neoplastic compound Indole-3-Carbinol @ 1µg/Kg body weight, whereas, group IV was given turmeric powder @ 300mg/Kg body weight and group V received turmeric in combination with garlic @ 150mg/Kg body weight from day one for four weeks continuously.

Weekly body weight was taken throughout the experimental period to observe the effects on growth rate. After four weeks of the treatment all the rats were sacrificed.

Tissue homogenate of liver and kidneys were prepared and subjected for assay of superoxide dismutase (SOD) by following the method of Mishra and Fridovich (1972), catalase activity (CAT) by Beers and Seezers (1952), protein by Lowery et al. (1951), lipid peroxidation product- malonaldehyde (MDA) by Esterbauer and Cheeseman (1993). Blood samples were subjected for the estimation of tissue marker enzymes like ALT, AST, ALP, Urea, and Creatinine by using Bayer's Auto pack kits procured from the market. The results were analysed statistically. Data was presented as mean± standard error (SE). The difference between groups was assessed using F-test (One way analysis of variance ANOVA). Gross pathological lesions of liver and kidneys were also studied at the end of the experiment.

3. RESULTS AND DISCUSSION

3.1 Body weight

In normal rats i.e. group I the mean values of body weight on zero day was 220.0 ± 1.2 and body weight in treated groups i.e. 225.3 ± 0.91 , 220.0 ± 1.34 , 220.2 ± 1.52 and 222.5 ± 1.25 in group II, III, IV and V respectively. The difference in total body weight gain was not found significant among all the groups, body weight in group II was found to be decreased than the body weight on zero day (Table 2).

Table 2: Average weekly body weight gain

Groups	Zero day	Week I	Week II	Week III	Week IV
I	220.0 ± 1.20	220.9 ± 0.10	222.6 ± 0.09	223.5 ± 0.87	224.9 ± 0.42
II	225.3 ± 0.90	225.1 ± 0.03	224.6 ± 0.06	223.6 ± 0.65	222.5 ± 0.67
III	220.0 ± 1.34	220.8 ± 0.45	222.3 ± 0.54	224.4 ± 0.56	226.8 ± 0.87
IV	220.2 ± 1.52	220.5 ± 0.13	221.0 ± 0.01	220.8 ± 0.09	222.1 ± 0.34
V	222.5 ± 1.25	222.5 ± 0.21	222.7 ± 0.87	222.8 ± 0.42	222.9 ± 0.68

The trend of average body weight gain in four weeks in group III, IV and V was observed to be lower than group I. Ramjee et al. (1992) reported that aflatoxin has been directly related to underweight status in children in Benin and Togo. Bedi et al. (1996) reported decrease in body weight in Guinea fowl fed on aflatoxin B₁. In present study the weight loss in group II may be due to the toxic and carcinogenic effects of DMBA.

3.2 Enzymatic Changes in Tissue Homogenates

The changes in MDA, SOD and CAT activity were studied in liver and kidney homogenates.

3.2.1 Liver homogenate

In normal rats fed on normal diet the mean value of MDA was 9.533 ± 0.163 nmol/L, which was raised by 53.2%, 4.72%, 15.28% and 18.54% in groups II, III, IV and V, respectively. There was significant increase in MDA level in liver tissue in DMBA treated group as compared to all the other groups. Indole-3- Carbinol has significantly lowered the MDA level in liver tissue. The treatment of turmeric and its mixture with garlic also showed the significant decrease in MDA level as compared to group II rats. The mean values of SOD in group I was found to be 47.55 ± 0.44 U/mg protein. There was significant decrease in SOD level in liver tissue in group II as compared to the normal and other treated groups as well. The treatment of turmeric alone and with garlic have shown the significant increase in SOD level in liver tissue as compared to DMBA treated rats but, level was found significantly lower than the normal and Indole-3- Carbinol treated groups. The catalase activity in liver tissue samples of normal group was 0.7933 ± 0.0361 U/mg protein. There was significant decrease in treatment groups. The trend was same as found in SOD activity (Table 3.0).

3.2.2 Kidney homogenate

The MDA level in kidney tissue of group I was 1.2885 ± 0.0503 nmol/L. There was significant increase in mean values of MDA in all the treatment groups as compared to normal. However, group III, IV and V have shown reversal effect, as the values of MDA were significantly lower than DMBA treated group II. Turmeric alone has shown better response than Indole-3- Carbinol. The mean values of SOD and CAT in group I were 47.55 ± 0.437 U/mg protein and 0.783 ± 0.007 Umg/protein respectively. The values of SOD and CAT in kidney tissue of all other treatment groups were significantly decreased. Indole-3-carbinol has shown good response to reverse the effects of DMBA. Although turmeric alone and with

garlic also showed significantly higher SOD and CAT activity of kidney tissue, but, it was significantly lower than Indole-3-Carbinol treated group (Table 3).

Table 3: Comparison of SOD, CAT and MDA in liver and kidney homogenates

Liver homogenate	MDA nmol/L	SOD U/mg protein	CAT U/mg Protein
Group I	9.533±0.163 ^a	47.55±0.440 ^a	0.7933±0.0361 ^a
Group II	14.61±0.331 ^c	31.50±1.87 ^b	0.5683±0.0261 ^c
Group III	9.983±0.149 ^b	37.50±0.89 ^c	0.78±0.0346 ^b
Group IV	10.99±0.452 ^d	36.49±1.83 ^d	0.652±0.0253 ^d
Group V	11.30±0.9392 ^d	35.67±1.53 ^d	0.66±0.0414 ^d
Kidney homogenate			
Group I	1.2885±0.0503 ^a	47.55±0.437 ^a	0.783±0.007 ^a
Group II	2.6633±0.0882 ^c	28.43±1.566 ^c	0.444±0.016 ^c
Group III	1.485±0.0364 ^b	43.80±0.9818 ^b	0.660±0.033 ^b
Group IV	1.227±0.0949 ^d	35.88±1.041 ^d	0.561±0.0145 ^d
Group V	1.950±0.0166 ^d	34.53±0.80 ^d	0.555±0.0224 ^d

(Values are mean ± SE) Figures with same superscript do not differ significantly ($P \leq 0.05$)

Pandey and Kumar (2007) reported the mean values for MDA, SOD, CAT and glutathione (GSH) in normal rats were 9.15 ± 0.19 nmol/L, 49.83 ± 0.7 U/mg protein, 0.80 ± 0.03 and 131.16 ± 1.44 U/mg protein respectively. Whereas the mean values of these antioxidant enzymes in liver of CCl_4 treated rats were 15.14 ± 2.32 nmol/L, 21.98 ± 0.57 U/mg protein, 0.031 ± 1.52 and 90.16 ± 1.01 U/mg protein, respectively. The study showed decreasing trend in enzymatic activity in the liver tissue in CCl_4 treated group. In present study also similar trend was found in DMBA treated group, which, establishes the toxic and carcinogenic effects of DMBA. Kuttun (2001) reported that daily administration of Curcumin @ 500mg for 7 days led to significant decrease in MDA. Activation of Kupffer cells contributes to liver injury by releasing cytotoxic agents, inflammatory cytokines and reactive oxygen species, which may lead to severe oxidative damage of liver cells (Wang et al., 2005). In present study the only DMBA injected rats have shown severe liver damage, which was significantly reversed by the turmeric.

3.3 Liver Function Test (LFT)

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes normally present in liver and heart cells. Alkaline phosphatase (ALP) is the enzyme made in liver, bone and the placenta. The higher levels of these enzymes indicate disease in bone, liver damage, bile duct obstruction or certain malignancies. In present study the levels of all these three enzymes were significantly elevated as compared to normal group I. The mean values of AST, ALT and ALP were significantly lowered by 22.5% , 21.4%, 19%, 29.16%, 26.43% and 25.3% in turmeric and turmeric plus garlic treated groups respectively as compared to DMBA treated group (Table 4) . Bedi et al. (1996), Hoon et al. (2006), Pandey and Kumar (2007) and Bedi et al. (2008) have also reported the increase in SGOT, SGPT, Acid phosphatase, ALP, urea and Creatinine due to hepatotoxicity induced by various

carcinogens in different experimental animals. The elevation of these enzymes could be attributed to the release of SGOT, SGPT and ALP from the cytoplasm in to blood circulation after rupture of the plasma membrane and cellular damage (Sabry et al., 2009). In present study the turmeric treated group has shown significantly lower levels of AST, ALT and ALP.

3.4 Renal Function Test (RFT)

The mean values of Creatinine and urea in group I were found to be 0.213 ± 0.025 and 9.981 ± 0.304 respectively , which were significantly increased by 194.23%, 50.7%, 101.88%, 144.13% and 201.67%, 14.32%, 102.58% and 115.0% respectively in groups II, III, IV and V. Groups treated with turmeric and turmeric with garlic have shown significant decrease by 31.39%, 17.03%, 32.85% and 28.73% in Creatinine and urea as compared to group II i.e. DMBA treated group (Table 4) .

Table 4: Comparison of mean values of AST, ALT, ALP, Urea and creatinine

Groups	AST U/L	ALT U/L	ALP U/L	Urea Mg/dl	Creatinine mg/dl
I	40.65±1.82 ^a	37.98±0.83 ^a	32.20±0.93 ^a	9.981±0.304 ^a	0.213±0.0250 ^a
II	77.80±1.50 ^c	79.33±1.37 ^c	89.78±1.79 ^c	30.11±1.322 ^c	0.627±0.0287 ^c
III	45.22±1.24 ^b	44.67±1.51 ^b	40.79±0.74 ^b	11.41±0.823 ^b	0.321±0.318 ^b
IV	60.29±1.53 ^d	64.26±0.97 ^d	66.05±0.92 ^d	20.22±1.35 ^d	0.430±0.0469 ^d
V	61.14±0.81 ^d	63.33±0.45 ^d	66.53±0.82 ^d	21.46±1.002 ^d	0.520±0.0374 ^d

(Values are mean ± SE) Figure with same superscript do not differ significantly ($p \leq 0.05$)

3.5 Gross Pathology of Vital Organs

The carcinogen mediated changes were observed in colour and size of liver of rats in DMBA treated group. Cyst formation in liver was observed in liver of group II rats.

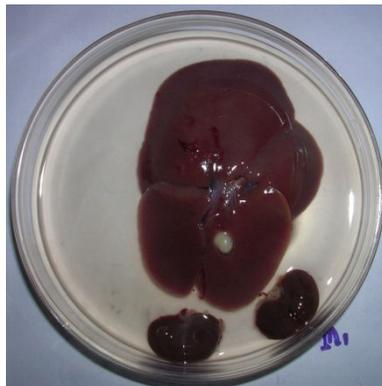


Fig. 1: Photograph of rat liver showing the presence of cyst in DMBA treated group



Fig. 2: Photograph of liver showing enlargement and paleness in rats compared with liver of normal group rats.

Rats treated with turmeric have not shown any cyst formation and enlargement in liver. But liver paleness was observed. In group V liver enlargement and paleness was observed without any cyst formation. Kidney and heart were found to be normal in all the groups. There was no change in colour and size of kidneys and heart (Figures 1 and 2). This study also provides supportive evidence for biochemical analysis.

4. CONCLUSION

Present study suggests that the turmeric have shown antioxidant, hepatoprotective nature as well as anti carcinogenic activity better than garlic. Further studies on the use of different levels of turmeric and garlic may be undertaken on different animal species to introduce these plants as hepatoprotective and anticarcinogenic for human beings also.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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