

## EFFECTS OF GARLIC AGAINST 7-12, DIMETHYL BENZANTHRACENE INDUCED TOXICITY IN WISTAR ALBINO RATS

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### ABSTRACT

In present study the antitoxic activity of garlic was studied in Wistar albino rats. The experimental animals were divided in to four groups each containing six animals. Group I served as normal control. All the other three groups, viz. II, III and IV were first challenged with 7-12 Dimethyl benzanthracene (DMBA). Therefore Whereas, group III and IV received Indole-3-Carbinol (1µg/kg) and garlic @ 300mg/kg body weight respectively, for four weeks. Group II however received no intervention. After four weeks, all the animals were sacrificed and the effects of the Indole-3-Carbinol and garlic were monitored by assaying the levels of lipid peroxidation (MDA), Superoxide dismutase (SOD) and Catalase (CAT) in liver and kidney homogenates. In addition Serum glutamate Oxaloacetate Transaminase (SGOT), Serum glutamate Pyruvate Transaminase (SGPT), Alkaline phosphatase (ALP), Urea and Creatinine were also studied.

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 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.

**Keywords:** Carcinogenic DMBA, hepatotoxicity, Garlic, SOD, CAT, MDA, SGOT, SGPT, ALP, Creatinine and Urea.

### INTRODUCTION

Our body is made up of cells that grow divide and then die in a predictable manner. Cancer occurs when something goes wrong in cells. As it grows, it may damage and invade nearby tissues. If a cancerous tumour grows, its birth place and moves on the place, it is referred to as metastasizing (Donna Mayers, 2006). Today, the Greek term carcinoma is the medical term for a malignant tumours derived from epithelial cells. It is Celcus who translated carcinos in to the latin, cancer, also meaning crab. Galen used "Oncos" to describe all tumours, the root for the modern oncology.

7,12- Dimethylbenzanthracene (DMBA) is a polycyclic aromatic hydrocarbon that causes tumour in the rats. DMBA is a well known mammary gland cancer initiator ( Amagese *et al.*, 1996 ). Phorbol ester 12- tetradecanoyl phorbol-13-acetate (TPA) is a very potent mouse skin tumour promoter (Vernon *et al.*, 1983). DMBA elicits immunotoxicity in the liver, spleen, thymus, 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 and Thurmond *et al.*, 1998).

Indole-3-Carbinol, a naturally occurring compound found in Cancer was induced in all the three groups (II, III, and IV) 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 garlic paste @ 300mg/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 SGOT, SGPT, 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.

### RESULTS AND DISCUSSION

Cancer is a disease characterised by a population of cells that grow and divide without respect to normal limits, invade and destroy adjacent tissues and may spread to distant anatomic sites through a process called metastasis. Cancer metastasis is a major cause of death. The present study is associated with the herbal therapy of cancer. The anticancer property of garlic was evaluated by measuring daily body weight gain per week, and also by monitoring the levels of lipid peroxidation malonaldehyde (MDA), Superoxide dismutase (SOD) and Catalase (CAT) in liver and kidney homogenates. Changes in SGOT, SGPT, ALP, Urea and Creatinine were also studied.

In normal rats i.e. group I the body weight was increased by 4.5% during the study period of four weeks. Whereas in group III and IV the increase in body weight was 3.09% and 1.45% respectively. In group II the body weight was decreased by 1.24% during the study period (Fig. 1.0). Although the values were not significantly different among all the groups, But there was decreasing trend in all the treated groups. The reduction in body weight of rats in group II might be due to carcinogenic effects of DMBA.

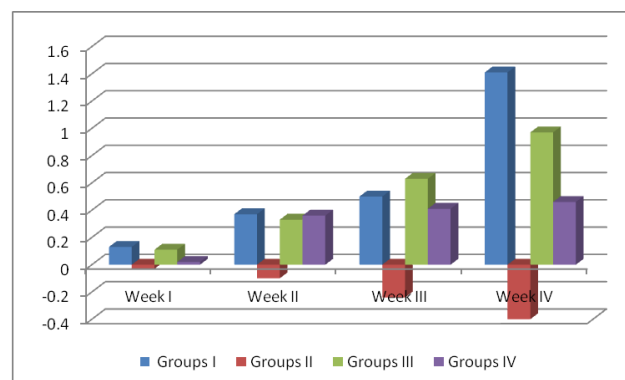


Fig 1.0: Average Per day Body weight gain

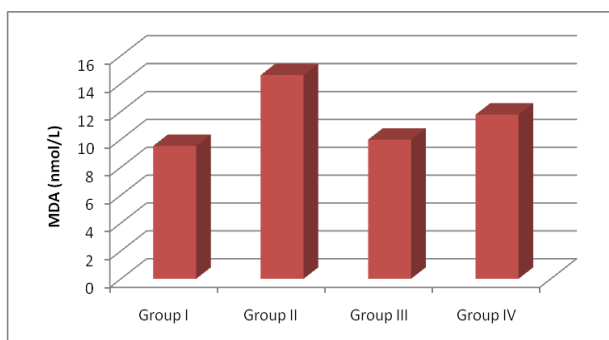
The toxic effects of DMBA on growth rate was reversed by Indole-3-Carbinol and garlic as the rats in these groups have shown gain in body weight. Similarly garlic juice depressed the toxicity in terms of body weight as compared to methyl mercury chloride treated rats, rate of increase was 17.5% in maternal body weight, 13.2% and 41.9% in fetal and litter's weight respectively (Lee *et al.*, 1999). On the contrary there were no significant differences in the final body weight of fish groups fed on diets with garlic. The maximum weight gain 19.8% was in group fed on garlic oil (M.A.A. Metwally 2009).

Similarly C. Canogullari *et al.*, 2010; Khan *et al.*, 2008 reported that garlic powder had no significant effect on egg weight and body weight of laying Japanese quail and hens. In present study the results are in consonance with previous studies, that garlic has no significant effect on body weight, but it was increased throughout the study period as compared to group II.

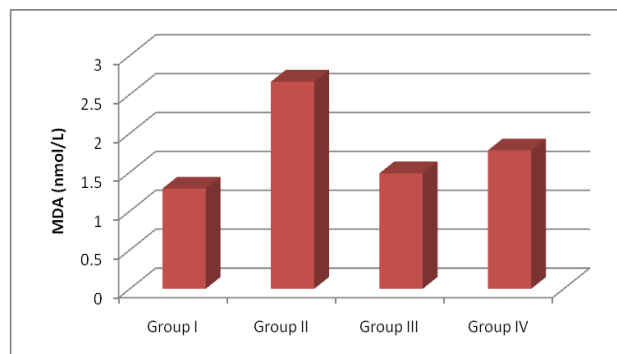
**Table 1: Distribution and details of the treatment**

Group	N	Type	Treatment
I	6	Normal Control	NIL
II	6	Negative Control	DMBA + TPA + Dexamethazone
III	6	Positive Control	DMBA + TPA + Dexamethazone + Indole-3-Carbinol
IV	6	Test I	DMBA + TPA + Dexamethazone + Garlic @ 300mg/Kg body weight

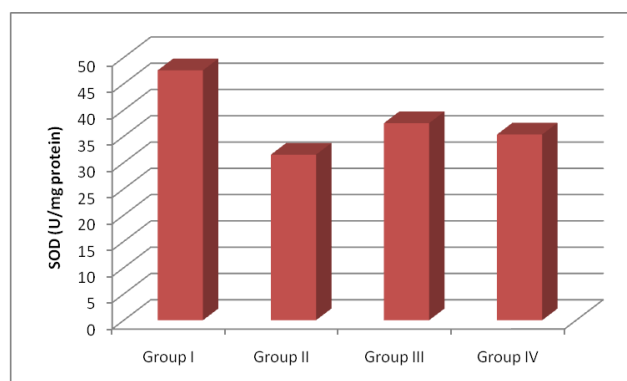
The values of MDA were increased significantly by 53.26%, 4.72% and 23.57% in liver tissue (Fig. 2.0) and 106.70%, 15.25% and 38.15% in kidney tissue homogenates of group II, III and IV respectively as compared to control (Fig. 2.1). Significant decrease in SOD and CAT values in liver tissue homogenate was observed by 33.75%, 14.09%, 25.68% and 28.36%, 1.68% and 18.69% in group II, III and IV respectively (Fig. 3.0 & 4.0). Similar trend of significant decrease in these enzymes was also observed in kidney tissue homogenate. The decrease in SOD and CAT was 40.21%, 7.89%, 36.49% and 43.30%, 15.71% and 31.03% in treatment groups II, III and IV respectively (Fig. 3.1 & 4.1). In the present study DMBA induced increased oxidative stress through the significant increase in MDA and significant decrease in SOD and CAT. This is in consonance with earlier observations reported by Sabry *et al.*, 2009. Decreased activity of the antioxidant enzyme (SOD), reflecting an oxidative stress state. Marked decrease in the protein content in hepatocyte's as well as haploidy was also proved. Bacterial endotoxin such as lipopolysaccharide is among the agents that cause immunological stimulation of Kupffer cells (Villavedra *et al.*, 1997). 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 garlic.



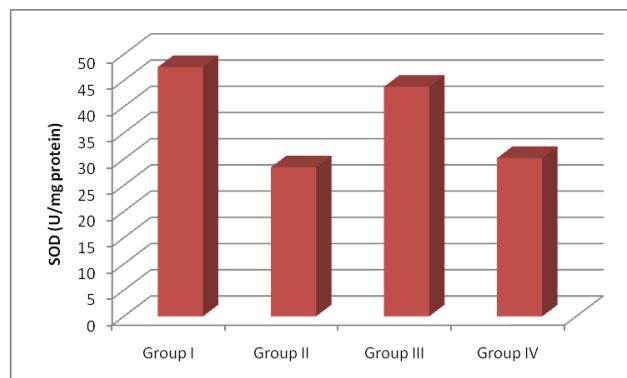
**Fig. 2.0 MDA Changes in Liver**



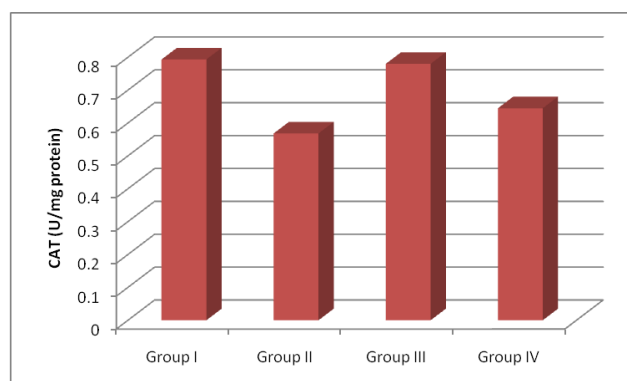
**Fig. 2.1 MDA changes in Kidney**



**Fig. 3.0 Changes in SOD levels in Liver**



**Fig. 3.1 Changes in SOD levels in Kidney**



**Fig. 4.0 Changes in CAT enzyme in Liver**

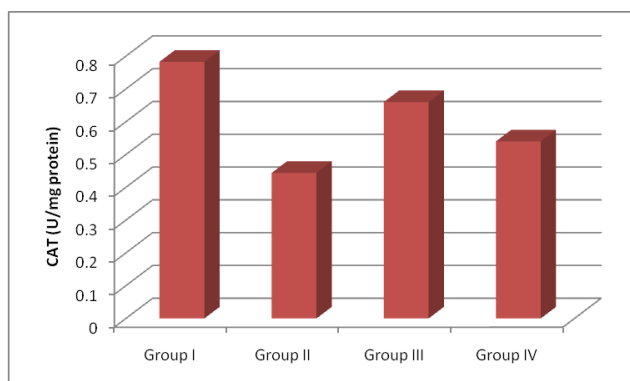


Fig. 4.1 Changes in CAT enzyme in Kidney

Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) are enzymes that are normally present in liver and heart cells. SGOT and SGPT are released in to blood when the liver or heart is damaged. Alkaline phosphatase (ALP) is enzyme made in the liver. Abnormally high values of ALP in blood may indicate disease in liver. In this study the levels of SGOT, SGPT and ALP in all the treatment groups i.e. II, III and IV were significantly increased by 91.39%, 11.24%, 55.13%, 108.87%, 17.61%, 71.09%, 178.81%, 26.68% and 110.71% respectively. Which indicates that liver was severely damaged in group II (Fig. 5.0). However the rats treated with garlic were able to reverse the carcinogenic effects of DMBA. The reversal effects of garlic towards DMBA induced toxicity were less than Indole-3-Carbinol. DMBA injected to rats lead to marked significant elevation in the levels of SGOT, SGPT and ALP, which are marker of hepatocellular damage. Creatinine is a breakdown product of creatine, which is an important part of muscle. A serum Creatinine test measures the amount of Creatinine in the blood. Creatinine and urea levels in blood increases if the kidney function is abnormal. In present study the Creatinine and urea levels were significantly elevated by 194.23%, 50.70%, 115.35%, 201.70%, 14.33% and 128.56% respectively in treatment groups II, III and IV as compared to control (Fig. 6.0 & 6.1). The significant increase in Creatinine and urea levels in blood indicates damage in kidneys due to DMBA induced toxicity.

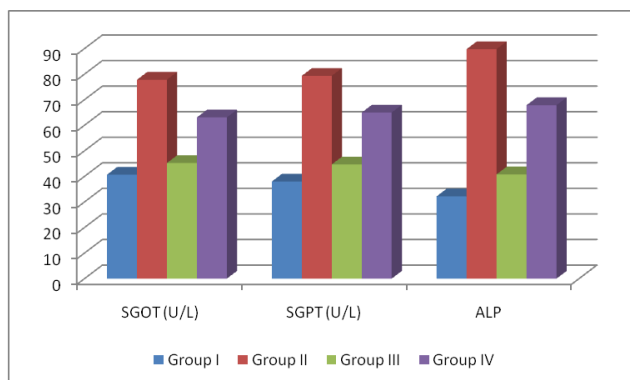


Fig. 5.0 Changes in Hepatic enzymes

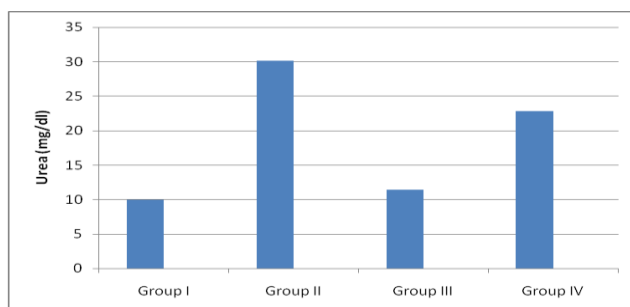


Fig. 6.0 Changes in Kidney Functions

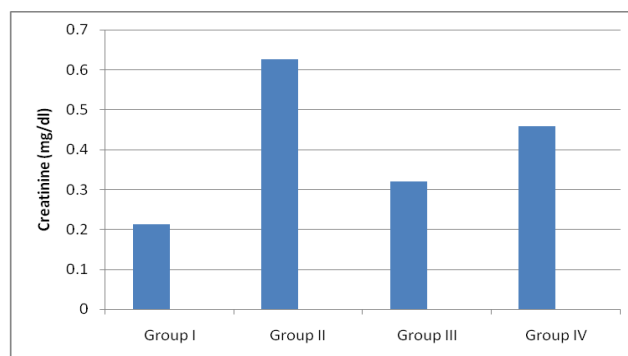


Fig. 6.1 Changes in Kidney Functions

Similar results i.e. increase in these enzymes have been observed when the rats were injected with N-nitroso diethyl amine (NDEA) and garlic has shown the hepatoprotective property by bringing down the levels of these enzymes. 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). The hepatoprotective property of garlic may be attributed to the presence of organosulphur compounds. Which have antioxidant and detoxifying properties. This detoxifying effect is explained by the induction of phase II antioxidant enzymes (Munday and Munday 2004). Garlic contains certain compounds such as germanium and selenium that play an important role in normalizing the oxygen utilization in the cells (Hussein *et al.*, 2007). The results of the present study proved significant renal and hepatoprotective nature of garlic.

#### 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. Rats treated with garlic have not shown any cyst formation and enlargement in liver. But liver paleness was observed. Kidney and heart were found to be normal in all the groups. There was no change in colour and size of kidneys and heart. This study also provides supportive evidence for biochemical analysis. Garlic in the present study seems to be a promising compound as it inhibits the damages to the kidney and liver tissues against oxidative damage and also preventing hepatic carcinoma induced by DMBA.

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