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Effect of Aqueous Extract of *Curcuma zedoaria* and *Gloriosa superba* Against DMH-Induced Colon Carcinogenesis In Wistar Rats.

A.M.Shaikh*[1,2], B.Shrivastava[2], K.G.Apte[1], S.D.Navale[3]

[1]APT Research Foundation, S. No 36/1/1; M. N. 199., Vadgaon Khurd, Pune (M.H.) India 411041,
[2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Rajasthan).
[3]BSDTAM Integrated Cancer Treatment and Research Center (ICTRC) Wagholi, Pune, (MH), India

Abstract: Cancer is the serious illness which can originate from any organ of the human body. The incidence and mortality rates of colorectal cancer are increasing statistically in recent decade and fourth most common type of cancer and second cause of death by cancer in the western world. The present study was conducted to evaluate in-vivo anti-colon cancer activity of aqueous extract of the rhizomes from *Curcuma zedoaria* and *Gloriosa superba* against 1,2-dimethyl hydrazine (DMH 40 mg/kg body weight) induced colon cancer in albino wistar rats. The result showed that administration of test drugs at dose of 5mg/kg exhibited significant ($p< 0.001$) anticancer effect when compared with standard drug Irinotecan at dose of 100 mg/kg body weight i.v once week four weeks.
Key words: DMH, Irinotecan, *Curcuma zedoaria* and *Gloriosa superb.*

Introduction:

Cancer is one of most serious health problem worldwide and associated with a problem of economical dimension with very high level of expenses. Cancer is a broad term used to encompass several malignant diseases.[1] There are several types of cancer affecting the different parts of body and each type of cancer is unique with its own causes, sign, symptom and various methods of treatment. Cancer is characterized by uncontrolled cellular growth with frequent cancer cell invasion to different body parts and spreading to other organ a process referred to as Metastasis. Metastasis is the major cause of cancer related mortality.[2]

Colon cancer is the third leading cause of cancer death in the United States. In china the incidence and mortality rates of colorectal cancer are increasing in recent decades being the fifth leading cause of cancer death.[3] Cancer risk are more increase in women today's world but still today from ancient time no exact treatment for cancer is available which will claim it will eradicate the cancer completely. The aim of anticancer agent is to trigger the apoptosis signaling system in the cancer cells while disturbing their proliferation.[4] 1,2-dimethyl hydrazine (DMH) is colon specific carcinogen known to produce free radical for circulation in experimental animal models. Free radicals or reactive oxygen species (ROS) are the main culprits in lipid per oxidation and cause destructive and irreversible damage to components of cell such as lipid, protein and DNA.[5] Antioxidant enzymes are the scavengers of free radicals and are modulated during carcinogenesis or after tumor formation. As a result of malignant state, alteration in the oxidant enzyme occur, if they are required for maintenance of the malignant state then recovery of decreased in enzyme could help to reverse the malignancy.[6]

Traditionally Curcuma zedoaria (family-Zingiberaceae) used for digestion, rheumatism, blood purification, skin disorders, hepatic protection and Gloriosa superba (family-Liliaceae) used to cure bruises sprains, Chronic ulcers, Haemorrhoids, Leproscy and impotenace.[6,7] Besides these plants has shown the many of biological activity like Anti-inflammatory, Anti-oxidant etc.[8]

Materials and Methods:

Chemicals and other drugs:

DMH was obtained from Sigma Aldrich and Irinotecan from Mac-Chem Products India Pvt. Ltd.

Collection and Authentication:

Rhizomes of plants *Curcuma zedoaria* and *Gloriosa superb*a were collected from western region of Maharashtra and authenticated by Botanical Survey of India (B.S.I) Pune, India.

Preparation of plant extracts:

The collected plant rhizomes were dried in shade for 2-3 days. The dried material was powdered by using grinder. The powdered plant materials were extracted with aqueous solvent using Soxhlet apparatus.

Phytochemical Screening:

Chemical screening was carried out on the extracts of powdered samples using standard procedures to identify the constituents as described by K. R. Khandelwal to test for the presence of saponins, tannins, flavonoids, coumarins, steroids and alkaloids.[9]

Acute toxicity study:

Acute toxicity study was performed as per OECD 423 guidelines.[10]

DMH-induced colon carcinogenesis:

30 animals were selected for colon cancer study, Group-I served as Normal control and first induction was done in 24 animals with DMH 40 mg/kg body weight was administered subcutaneously (s.c) to the rats of corresponding groups at twice a week for 2 week and dosing were started one week after last dose of DMH and continued till the termination of experiment. These animals were randomized into 4 groups of 6 animals each. Group-II served as Colon cancer control, Group-III served as Colon cancer with Irinotecan 100 mg/kg iv, Group-IV served as Colon cancer with 5mg/ kg *Curcuma zedoaria* extract and Group-V served as Colon cancer with 5mg/ kg *Gloriosa superba* extract.

Rats were sacrificed at 16 weeks of experimental time. Blood was collected, serum were separated by centrifugation which was used for the estimations of Creatinine, SGPT, SGOT, ALP, LDH. Enzymatic assays were performed by isolating the liver and colon. The homogenate of liver and colon were prepared and assays like Catalase (CAT), Lipid peroxidation (LPO) and Reduced glutathione content (GSH) were conducted. Sections of Colon, Caecum, Liver and Kidney were fixed with 10% formalin for histological analysis.[11]

Figure No. 1 Colon cancer images.

Histopathological studies:

The isolated tissue pieces of Colon, Caecum, Liver and Kidney were sliced into 5 mm pieces and fixed into neutral formalin (10%) solution for 3 days. Colon, Stomach, Caecum, Liver and Kidney pieces were washed under running water for about 4 hrs to remove the preservative. This was followed by dehydration with alcohol of ascending grade (50%, 70 %, 80%, and 90%) for 2 hrs each. Final dehydration was carried out using absolute alcohol with two changes of 1hour. Cleansing was done by using xylene with changes at 1 hour. After cleansing the tissue sections were subjected to paraffin infiltration in automatic tissue processing unit for histological analysis.[12]

Results and Discussion:

Phytochemcal screening of aqueous plant extracts of *Curcuma zedoaria* (CZ) and *Gloriosa superba* (GS) were shows the presence of alkaloids, saponins, tannin and phenolic compounds.

Acute toxicity study were performed as per OECD 423 guidelines, the Aqueous extract of the plants *Curcuma zedoaria* (CZ) and *Gloriosa superba* (GS) has shown no any toxic effect at the dose of 50 mg/kg body weight.

The anticancer potential of the aqueous plant extract of *Curcuma zedoaria* and *Gloriosa superba* were evaluated on DMH-2Hcl induced colon carcinoma in rats. The results showed that administration of test drugs at the dosage of 5 mg/kg body weight exhibited significant (***p<0.001) anticancer effect when compared with standard drug Irinotecan at dose 100 mg/kg body weight i.v. The parameters like SGPT, SGOT, ALP, LDH, Creatinin, Catalase, LPO and GSH were evaluated in colon cancer model. The significant (***p<0.001) activity shown by aqueous plant extract of *Curcuma zedoaria* at the dosage of 5 mg/kg body weight when statistically compared with standard drug Irinotecan at dose 100 mg/kg body weight i.v using one way ANOVA followed by Dunnett's test. Where the LDH (628.2±111.8**), LPO (0.25±0.024***) and GSH (0.34±0.002***) shows statistically significant values. The aqueous plant extract of *Gloriosa superba* also shown significant activity at the dosage of 5 mg/kg body weight when statistically compared with standard drug Irinotecan at dose 100 mg/kg body weight i.v using one way ANOVA followed by Dunnett's test. Where the LDH (438.3 ±114.5***) LPO (0.44±0.054***) and GSH (0.34±0.002***) shows statistically significant (***p<0.001) values.

Histopathologically aqueous plant extract of *Curcuma zedoaria* at the dosage of 5 mg/kg body weight reveals protective effect (mild changes) compared with standard Irinotecan at dose 100 mg/kg body weight i.v.

Table No. 1 Effect on various biomarkers by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*

Parameter	NC	DC	STD	T-I (CZ)	T-II (GS)
SGPT(U/L)	63.86 ±2.852	91.30 ±17.15	67.44 ±8.755	77.74 ±8.959	73.49 ±3.178
SGOT(U/L)	150.7 ±6.979	172.8 ±13.23	152.2 ±9.951	162.0 ±12.02	168.7 ±20.58
ALP(U/L)	371.2 ±19.81	612.0 ±42.46	391.3 ±27.04**	536.7 ±78.62	483.0 ±42.13
LDH(IU/L)	169.8 ±6.384	1087 ±77.68	276.8 ±50.09***	628.2 ±111.8**	438.3 ±114.5***
Creatinine(mg/dl)	0.83±0.042	1.05±0.056	0.85±0.061	0.95±0.125	0.98±0.037
Catalase(mMol/gm)	0.62±0.048	0.34±0.004	0.45±0.004*	0.35±0.016	0.47±0.017*
LPO(nMol/gm)	0.38±0.002	0.77±0.005	0.24±0.013***	0.25±0.024***	0.44±0.054***
GSH-L(uMol/gm)	0.33±0.001	0.25±0.008	0.36±0.005***	0.34±0.002***	0.34±0.002***

Values are Mean ± S.E.M., n=6 in each group, *p<0.05, **p<0.01, ***p<0.001 when compared with disease control group (One way ANOVA fallowed by Dunnett's test).

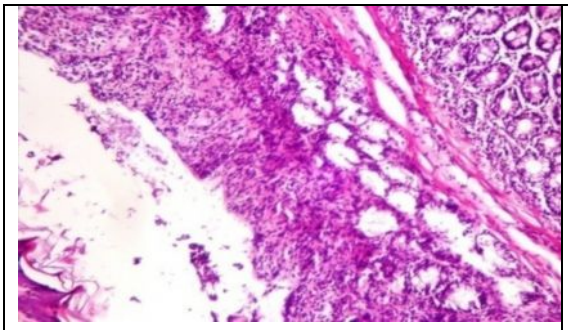
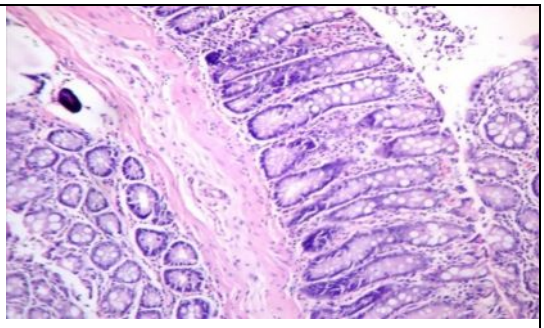
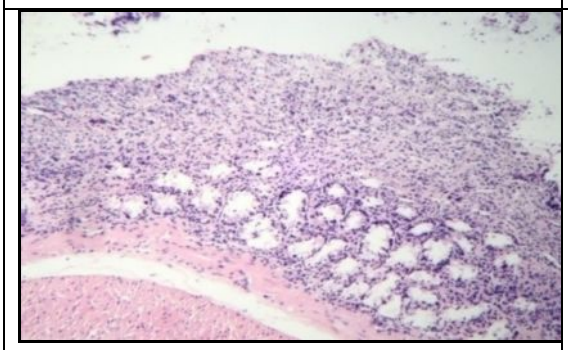
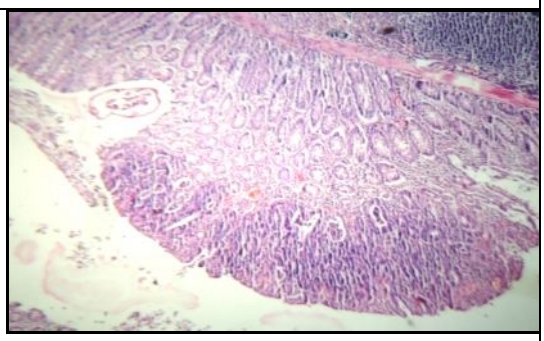
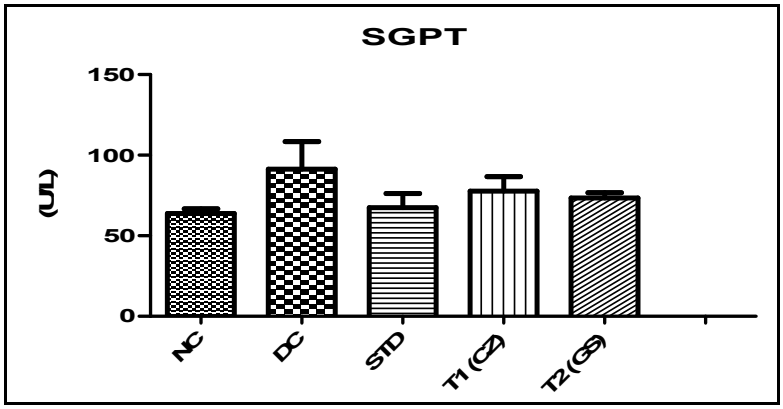
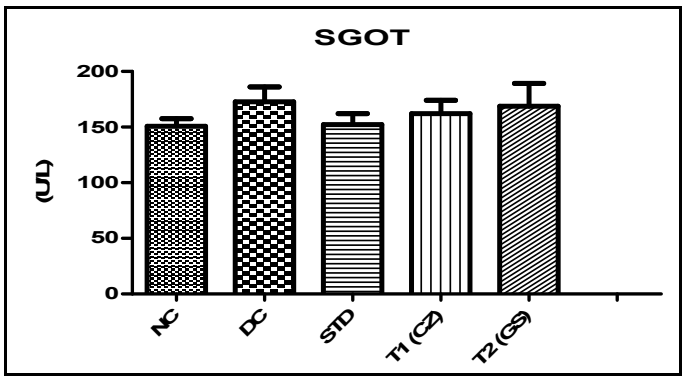
	
DC: Proliferation of Tumour cells and inflammatory growth in mucosa. Absence of normal villi in mucosa.	**STD (Irinotecan 100 mg/kg):** Microphotograph showing normal mucosa with villi and glandular features and muscularis mucosa.
	
Test-1(CZ): Colon showing normal mucosa with mild Proliferation, glandular epithelium and submucosa with muscularis mucosa.	**Test-2(GS):** Section showing mild degenerative changes of glands in submucosa and proliferative changes with infiltration of few inflammatory and neoplastic cells.

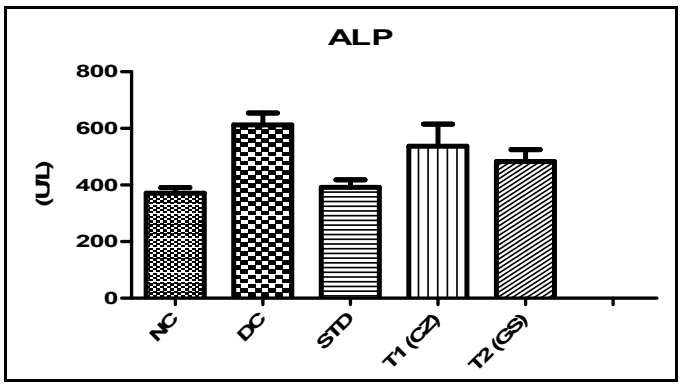
Figure No. 2 Histopathological examination of Colon tissues.



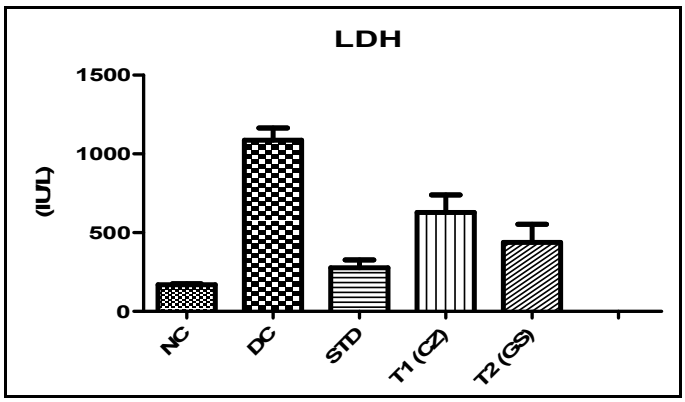
Graph No. 1 Effect on SGPT by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



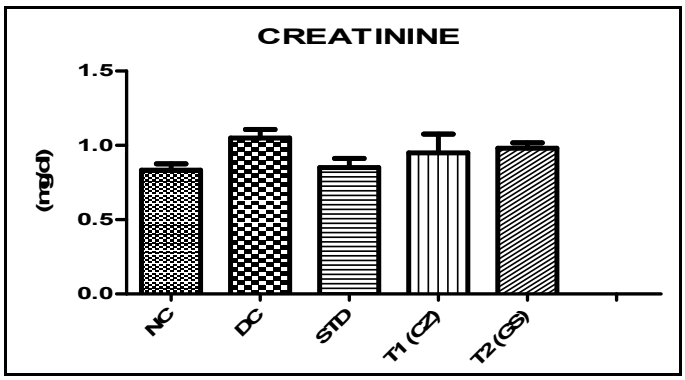
Graph No. 2 Effect on SGOT by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



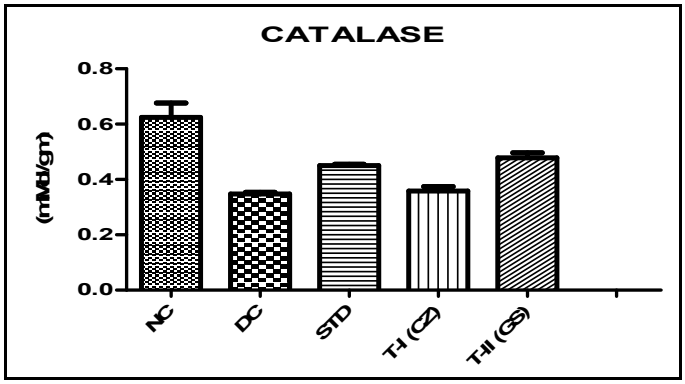
Graph No. 3 Effect on ALP by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



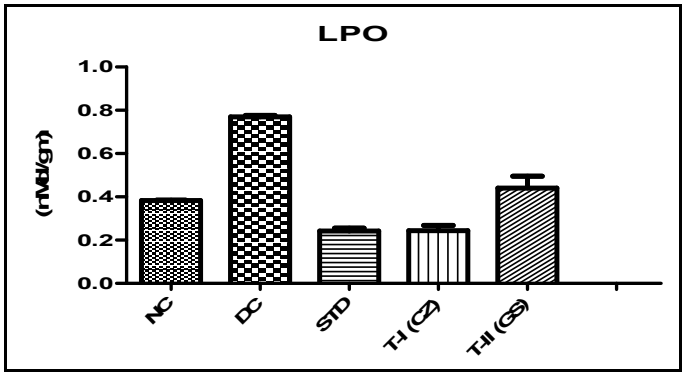
Graph No. 4 Effect on LDH by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



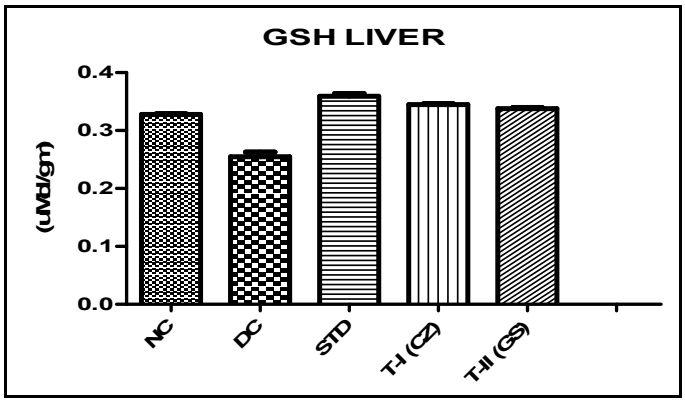
Graph No. 5 Effect on Creatinine by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



Graph No. 6 Effect on Catalase by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



Graph No. 7 Effect on LPO by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



Graph No. 8 Effect on GSH liver by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*

Conclusion:

From the results obtained in this study, it can be concluded that the aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba* at dose 5mg/ kg body weight has modified the levels of biomarkers and shown some moderate histopathological changes, which reveals that *Curcuma zedoaria* and *Gloriosa superba* possess significant anticancer activity against DMH induced colon carcinogenesis when compared to standard drug Irinotecan at dose of 100 mg/kg body weight.

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