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Research Article

Comparative Anticancer Evaluation of *Curcuma zedoaria* and *Gloriosa superba* against 7, 12-Dimethylbenz [*a*] anthracene (DMBA) Induced Mammary Tumors in Rats

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Abstract
In this study tumors were developed with carcinogenic agent 7-12 Dimethylbenz[a] anthracene (DMBA) in female albino wistar rats at a dose of 7.5 mg/kg sc. once in a week for 4 weeks and waited for 90 days considering from first day of initiation until development of tumors. Once tumors were developed at 90 days then tumor size was measured weekly with digital vernier caliper for four weeks during the treatment of plant extracts. Plant extracts of *Curcuma zedoaria* and *Gloriosa superba* comparatively evaluated for their potential to reduce tumor size against Paclitaxel. Administration of aqueous extracts of *Curcuma zedoaria* and *Gloriosa superba* at dose of 5mg/kg body weight orally for 30 days was associated with significant decrease in tumor volume as compare to that of Paclitaxel which was given at dose of 1mg/kg body weight i.p. Apart from this *Curcuma zedoaria* and *Gloriosa superba* aqueous plants extracts treated groups has shown significant down regulation of biological markers like SGPT, ALP, LDH, LPO, Urea, and up regulation of GSH, Total protein. Therefore *Curcuma zedoaria* and *Gloriosa superba* when administered orally can act as effective curative agent towards DMBA induced mammary tumors.

Keywords: *Curcuma zedoaria, Gloriosa superba*, Mammary tumors, 7-12 Dimethylbenz [a] anthracene.

1. Introduction
The use of medicinal plants for the treatment of various ailments is associated to folk medicine which is used by people from different parts of world.[1] Nature has provided abundant plant source as a remedy to cure all diseases of mankind. As a result majority of population of world largely relies on plants store house owing to its natural origin and lesser side effects.[2,3] The plants of ginger family Zingiberaceae most widely used in traditional system of medicine.

Curcuma zedoaria belongs to family: Zingiberaceae also known as Krachura in Sanskrit Gandamatsi in hindi and Sutha in Bengali.[4] Traditionally *Curcuma zedoaria* used for the treatment of digestion, rheumatism, blood purification, skin disorders and in hepatoprotection. Beside this scientific activity like antibacterial, antimicrobial, analgesic, anti-inflammatory and antidiabetic were carried out on this plants.[5-7]

Plant from liliaceae family *Gloriosa superba* commonly known as Langli in Sanskrit, in Marathi it is known as Kal-lavi and in English it is known as Glory lily. Traditionally it is used for treatment of bruises and sprains, chronic ulcer, haemorrhoids, cancer, leprosy and

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impotence. Previously carried scientifically activity like antianxiety, antimicrobial, antihelmintic and hepatoprotive activities on this plant. In present study comparative evaluation of anticancer potential of *Curcuma zedoaria* and *Gloriosa superba* evaluated pharmacologically.[8-10]

Materials & Methods

Plant material and extraction

The plant material were collected from north-west region of Maharashtra and authenticated in Botanical survey of India in Pune. Rhizomes of *Curcuma zedoaria* and *Gloriosa superba* were separately sliced and air dried in shade for 120 hrs then grinded into a course powder and extracted with aqueous solvent using soxhlet extraction method.

Chemicals and other drugs

DMBA was obtained from Sigma Aldrich and Paclitaxel from Joras oncology Pvt. Ltd. Thane, Maharashtra.

Animals

The institutional animal ethics committee (Register No. 40/CPCSEA/1999), National Toxicology Centre, Pune, India approved the experimental design (Proposal No.44/1415 dated 06/10/2014). Albino (Wistar) female rats of 200-250g used for study. Animals were housed in well ventilated room (temperature 22 ± 2^0C, humidity 40-60% and 12h light/dark cycle) at National Toxicology Centre. Animals were fed with standard pellet diet and water. All studies were conducted in accordance with Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines “Guide for care and use of Laboratory Animals.”

DMBA-induced experimental design of Breast cancer

In this model 30 female albino wistar rats were selected out of that induction was in 24 animals with the carcinogenic chemical 7-12 dimethylbenz (a) anthracene at the dose of 7.5 mg/kg subcutaneously once a week for four weeks 7.5 mg/kg of 7, 12–dimethylbenz(a) anthracene (DMBA) was dissolved in 1ml of vehicle (0.5ml of sunflower oil + 0.5 ml of saline) and injected by subcutaneous injection beneath the mammary gland at right side, waited for 90 days till the visualization of tumors. There were tumor yield after 90 days with the initiation of DMBA. Tumors size were measured using digital Vernier caliper and animals were randomized in even number and size on basis of tumor volume in different groups of six rat each as Group I- Served as control group, Group II – Served as disease control, Group III- Served as standard group with paclitaxel (1mg/kg body weight i.p) once in a week for 4 weeks, Group IV-Treated with 5mg/kg aqueous extracts of *Curcuma zedoaria* (P.O) for 30 days, Group V- Treated with 5mg/kg aqueous extracts of *Gloriosa superba* (P.O) for 30 days.

During the 30 days treatment all tumors size were measured weekly before and after treatment (7, 14, 21, 28 days) by digital vernier caliper. At the end of the experiment i.e. after 120 days all the animals were sacrificed, blood was collected serum was separated by centrifugation which was used for the estimations of Urea, Glucose, SGPT, ALP, LDH. Enzymatic assays were performed by isolating the liver and mammary gland. The homogenate of liver and mammary gland were prepared and assays like Catalase (CAT), Lipid peroxidation (LPO), reduced glutathione content (GSH) were conducted. Histopathological examination of breast, liver and kidney were done.[11,12]

Histopathological studies

The isolated tissue pieces of growths of mammary gland, liver and kidney were sliced into 5 mm pieces and fixed into neutral formalin (10%) solution for 3 days. Breast, 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 01 hour. After cleansing the tissue sections were subjected to paraffin infiltration in automatic tissue processing unit.

Results and Discussion

The present study was carried out to evaluate the anticancer potential of the aqueous plant extract of *Curcuma zedoaria* and *Gloriosa superba* on DMBA induced mammary carcinoma in female wistar albino rats. The

result showed that administration of test drugs at dosage of 5mg/ kg body weight exhibited enhanced anticancer effect when compared with standard drug Paclitaxel at dose of 1mg/kg body weight (Table No. 1). The parameters like total protein, SGPT, ALP, LDH, Glucose, Urea, Catalase, LPO and GSH were evaluated in breast cancer model (Graph No.1-11).

The significant ($p<0.001$) activity shown by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba* at the dosage of 5mg/ kg body weight when statistically compared with standard drug Paclitaxel at dose of 1mg/kg body weight using one way ANOVA followed by dunnett's test where the lower significant p value is $p< 0.05$ and higher significant p value is $p<0.001$. In addition the tumor volume data reflects the potential antitumor activity of *Curcuma zedoaria* and *Gloriosa superba* aqueous plant extracts where antitumor activity of *Gloriosa superba* is to be prominent one at the dosage of 5mg/kg body weight (Table No. 2). Histopathologically aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba* exhibited good effect when compared with standard drug Paclitaxel at dose 1mg/kg body weight (Figure No. 3).

Conclusion

Aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba* at dose 5mg/ kg body weight has shown significant potential to reduce tumor volume against DMBA induced breast carcinogenic tumor when compared to standard drug Paclitaxel at dose of 1mg/kg body weight.

Acknowledgement

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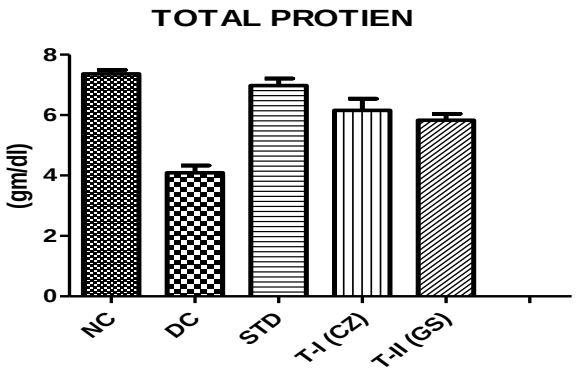
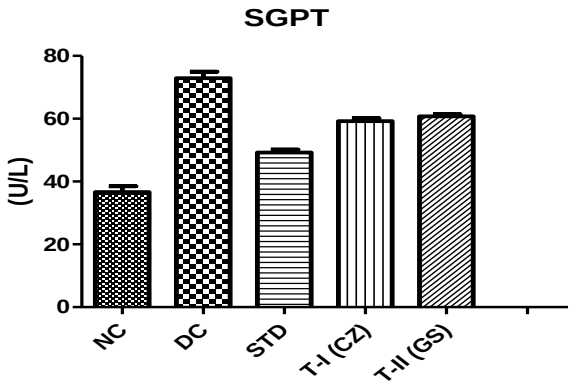
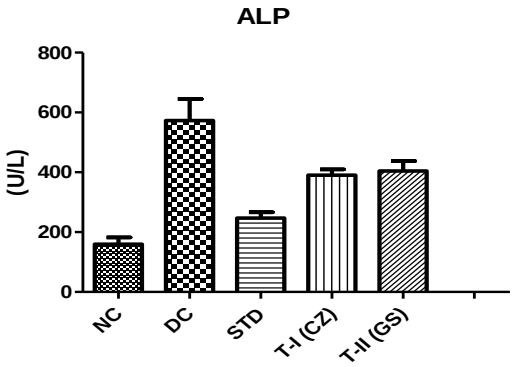
Graph No. 1 Effect on total protein by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



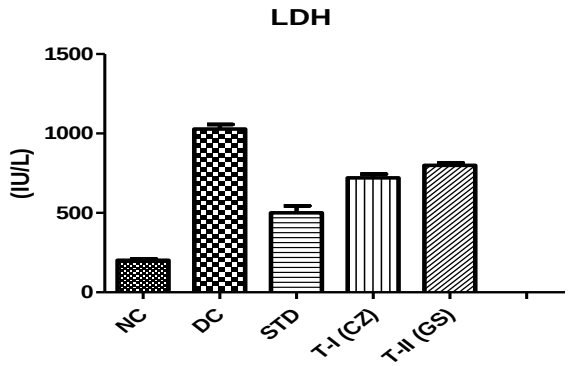
Graph No. 2 Effect on SGPT by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



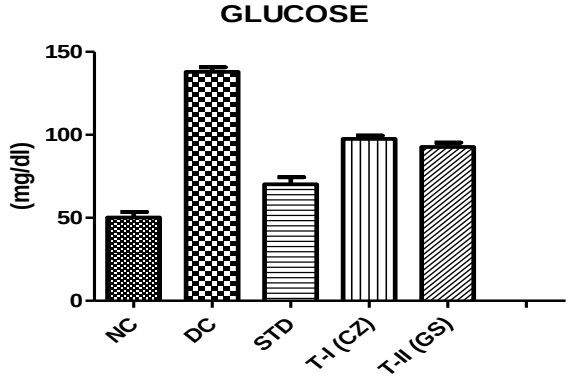
Graph No. 3 Effect on ALP by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



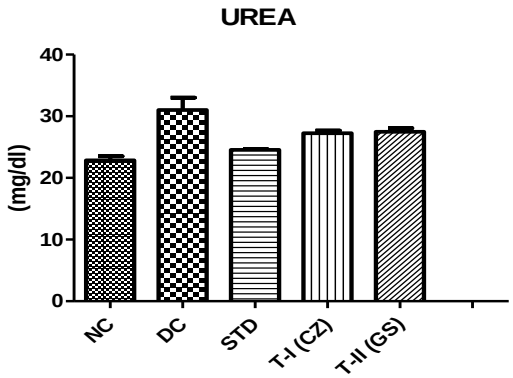
Graph No. 4 Effect on LDH by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



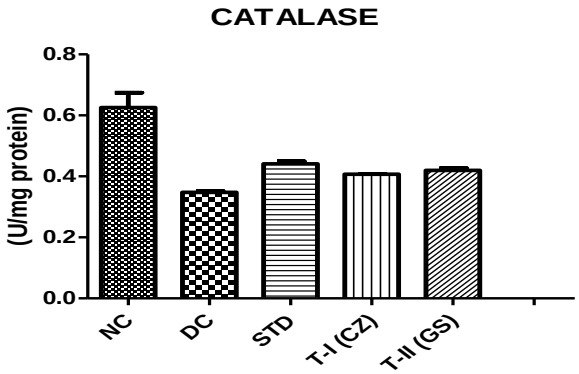
Graph No. 5 Effect on Glucose by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



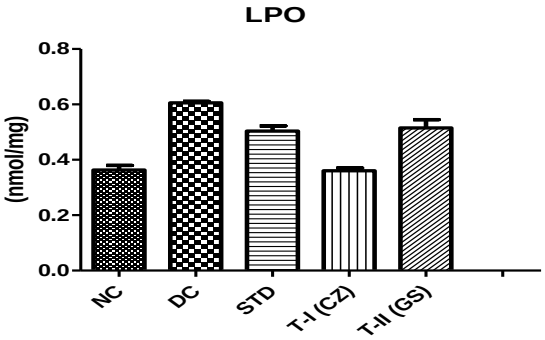
Graph No. 6 Effect on Urea by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



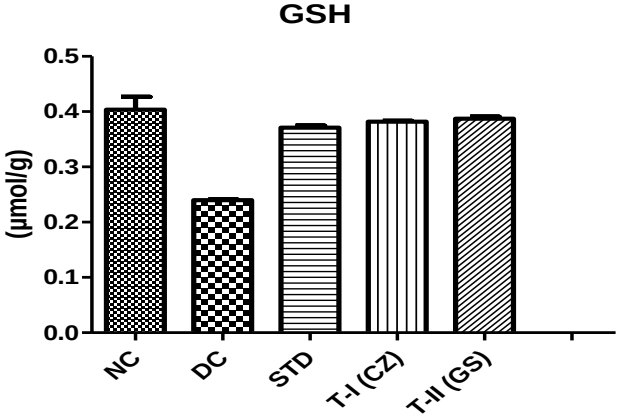
Graph No. 7 Effect on Catalase by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



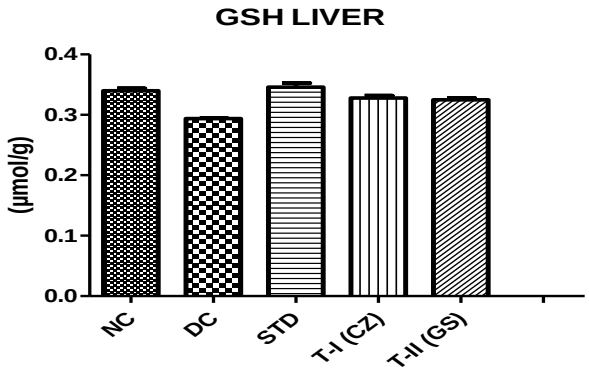
Graph No. 8 Effect on LPO by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



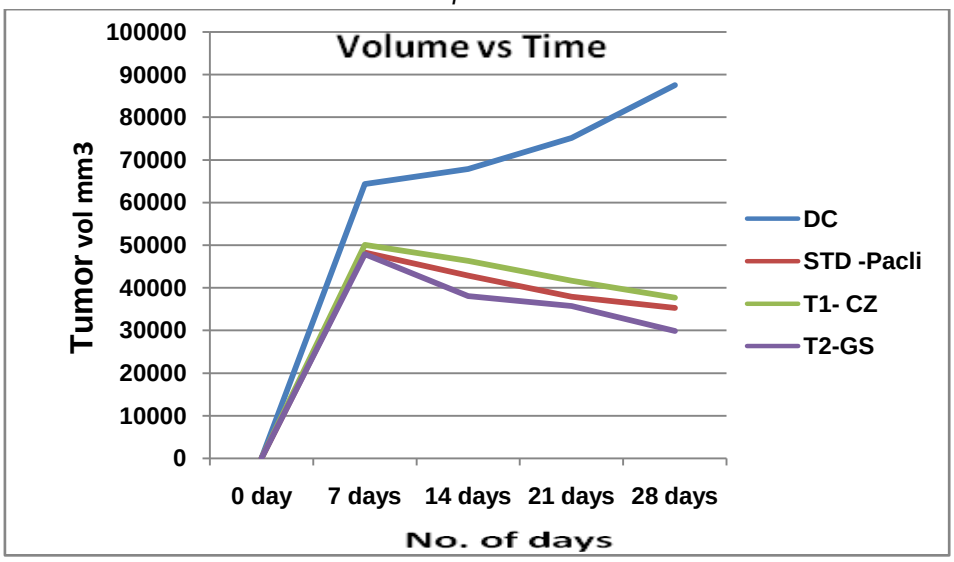
Graph No. 9 Effect on GSH by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



Graph No. 10 Effect on GSH liver by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



Graph No. 11 Effect on Tumor Volume by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*



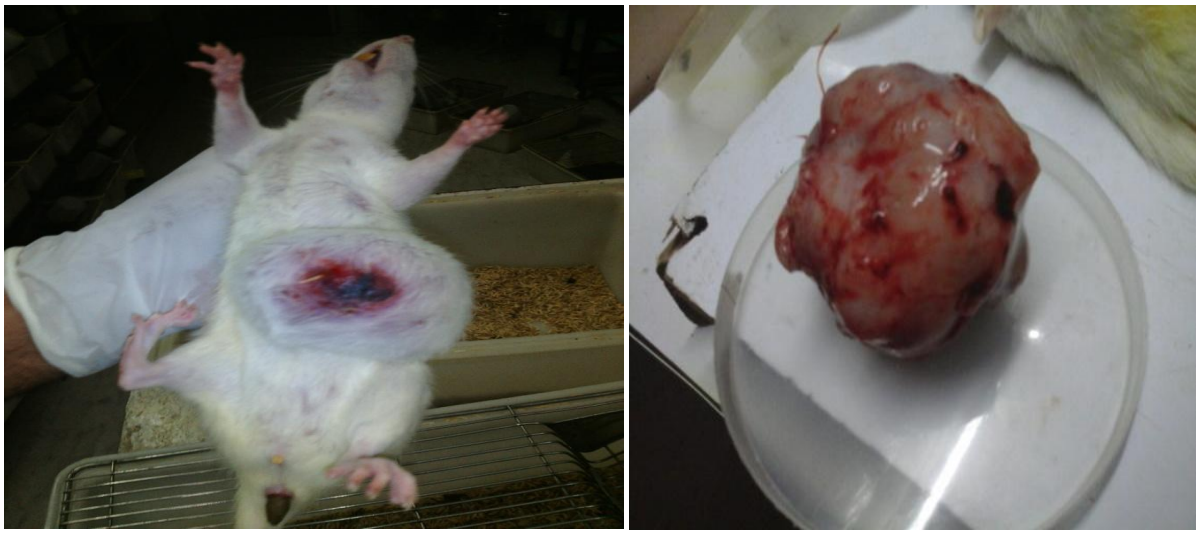


Figure 1- Developed Breast Tumor.

Figure 2- Measurement of Breast Tumor.

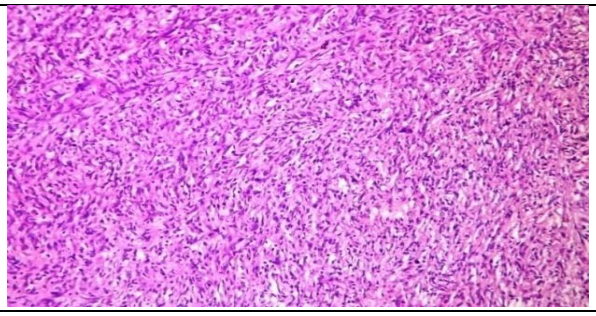
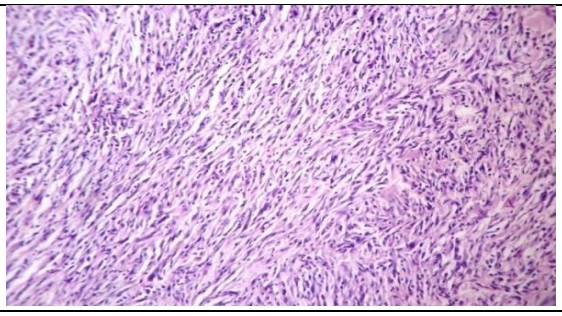
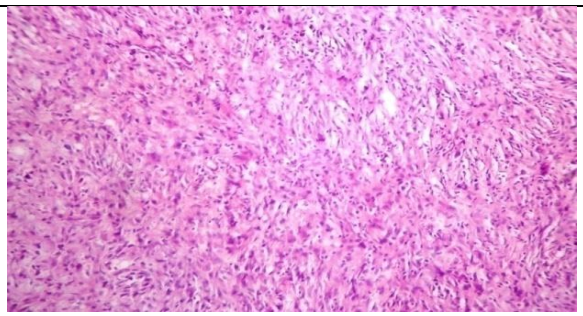
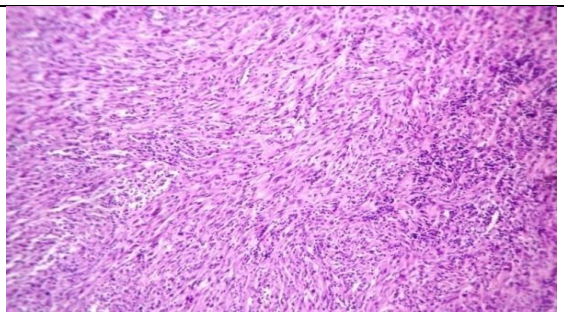
	
DC: Proliferative neoplastic cells with pleomorphisim of cells and basophilia of nucleus of tumour cells**.**	**STD (Paclitaxel 1mg/kg):** Proliferative neoplastic cells with less compact arrangement (as compared to tumours of disease control)
	
Test-1(CZ): Neoplastic cells with loss of compactness and degenerative and necrotic changes in tumour mass. Note that the tumour cells are less densely arranged as compared to DC.	**Test-2(GS):** Proliferative neoplastic cells. Note that the tumour cells are less densely arranged as compared to DC.

Figure 3- Histopathological examination of Breast.

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)
Total protein (gm/dl)	7.36 ± 0.134	4.09 ± 0.234	6.97 ± 0.230***	6.15 ± 0.385***	5.82± 0.218***
SGPT (U/L)	36.61 ± 1.870	72.88 ± 2.109	49.22 ± 0.942***	59.26 ± 0.917***	60.76 ± 0.684***
ALP (U/L)	159.7 ± 22.77	573.2 ± 71.50	246.7 ±19.43***	390.7 ± 18.83**	404.0 ± 33.85**
LDH (IU/L)	201.8 ± 8.324	1029 ± 27.91	501.2 ± 43.40***	720.3 ± 24.12***	799.2 ± 16.93***
Glucose (mg/dl)	50.17 ± 3.439	137.9 ± 2.709	70.17 ± 4.324***	97.50 ± 2.029***	92.67 ± 2.704***
Urea (mg/dl)	22.85 ± 0.672	31.01 ± 1.995	24.57 ± 0.113***	27.24 ± 0.432*	27.47 ± 0.599*
Catalase (U/mg protein)	0.62 ± 0.048	0.34±0.004	0.44±0.008*	0.40±0.001 ^{ns}	0.41±0.007 ^{ns}
LPO (nmol/mg)	0.36 ± 0.041	0.60±0.005	0.50±0.018**	0.36±0.009***	0.51±0.029**
GSH-B (µmol/g)	0.40 ± 0.057	0.23±0.001	0.37±0.004***	0.38±0.001***	0.38±0.003***
GSH-L (µmol/g)	0.33±0.003	0.29±0.007	0.34±0.006***	0.32±0.003***	0.32±0.003***

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).

Table No. 2- Effect on Tumor Volume by aqueous plant extracts of *Curcuma zedoaria* and *Gloriosa superba.*

Groups	0-7 Days			0-14 days		
	Before treatment	**After treatment**	**% Volume**	**Before treatment**	**After treatment**	**% Volume**
DC	62166.57	64331.76	-3.48	62166.57	67861.18	-9.16
STD (Pacl)	54280.09	48254.58	11.1	54280.09	42817.19	21.11
T-I (CZ)	51185.2	50079.1	2.16	51185.2	46298.76	9.54
T-II (GS)	52255.77	47903.16	8.32	52255.77	38095.53	27.09

Groups	0-21 Days			0-28 Days		
	Before treatment	**After treatment**	**% Volume**	**Before treatment**	**After treatment**	**% Volume**
DC	62166.57	75160.3	-20.9	62166.57	87538.01	-40.81
STD(Pacl)	54280.09	37917.88	30.14	54280.09	35279.89	35
T-I (CZ)	51185.2	41665.88	18.59	51185.2	37692.59	26.36
T-II (GS)	52255.77	35730.22	31.62	52255.77	29897.64	42.78

Source of Support: Nil.

Conflict of Interest: None declared

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