Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(2); 383-389

ISSN: 0975-4873

Research Article

Protection Against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and *In vitro* Anti-arthritic Potential of *Costus speciosus* Rhizome Extract.

Kala Chandra[1], Ali Syed Salman[2*], Abid Mohd.[2], Rajpoot Sweety[1], Khan Najam Ali[2]

[1]*Department of Pharmaceutical Technology, Meerut Institute Engineering and Technology, NH 58, Bypass Road, Baghpat Crossing, Meerut – 250005, Uttar Pradesh, India*
[2]*School of Pharmaceutical Sciences, IFTM University, Lodhipur rajput, Delhi road (NH-24), Moradabad – 244102, Uttar Pradesh, India*

Available Online: 31st March, 2015

ABSTRACT

Objective: Oxidative stress and oxidative stress induced DNA damage is one of the potential cause of arthritis. Methanolic extract of *Costus speciosus* rhizome is rich in flavonoids. Flavonoids are very well known to reduce oxidative stress. Reduction in oxidative stress may be the reason of inhibition of DNA damage. The objective of research is to study the effect of *Costus speciosus* rhizome extract against oxidative stress induced DNA damage as well as its in-vitro anti-arthritic potential. Methods: Extraction of *Costus speciosus* rhizome was done with 80% methanol using soxhlet assembly. Quantitative estimation of extract for flavonoids was done. Arthritis was induced by FCA administration. Different oxidative stress parameters like nitric oxide, GSH, MDA were evaluated. DNA estimation was done to measure oxidative stress induced DNA damage. *In vitro* anti arthritic activity was also evaluated. Results: *Costus speciosus* rhizome extract (100 mg/kg , 200 mg/kg), significantly reduced FCA induced oxidative stress as it reduced NO level, MDA level and raised GSH level in comparison to arthritic control ($p<0.05$) prophylactically and therapeutically. *Costus speciosus* rhizome extract significantly inhibits DNA damage in comparision arthritic control at $p<0.05$ prophylactically and therapeutically. *Costus speciosus* rhizome extract (100 mcg/ml and 200 mcg/ml) inhibits protein denaturation, proteinase action and membrane destabilization in comparision to control at $p<0.05$ indicating its antiarthritic potential. Conclusion: *Costus speciosus* rhizome extract affords protection against FCA induced oxidative stress and DNA damage. It also possesses anti-arthritic potential. These effects might be mediated via anti-oxidant potential.

Keywords: *Costus speciosus*, anti-arthritic activity, DNA damage.

INTRODUCTION

Rheumatoid arthritis (RA) is common auto-immune persistent inflammatory arthritis, occurring throughout the world and ethnic group. This is the most marked in those with severe disease, with reduction in expected lifespan by 8-15 years[1]. Oxidative stress is known to be one of the various potential causes of rheumatoid arthritis. Moreover, there are finding indicating that DNA damage level increases in patient with RA. Elevated DNA damage may be associated with increased oxidative stress and decreased anti-oxidant capacity[4]. NSAIDS, DMARDS and combination of both are preferred medication for present scenario. But, adverse effect attributable to use of Disease modifying antirheumatic drugs (DMARDs) and Non-steroidal anti-inflammatory drugs (NSAIDS), particularly in aged patient, has dragged their attention for alternative medication[3]. Complementary and alternative medicine (CAM) is most preferred option to cure this disease. It was found that about 60-90% of dissatisfied patients prefer CAM. Herbal and chiropractic therapies are widely used treatment as they are reported to have minor adverse effect[2].

It is mentioned in literature that rhizome of *Costus speciosus* Koen is traditionally used for treating inflammatory condition like arthritis. *Costus speciosus* Koen. (Keu, Crape ginger), an Indian ornamental plant, belonging to family Costaceae. The plant is widely distributed in India in the tropical or sub-tropical climate from the sea level to the Himalayas, excluding the arid and semi-arid areas of Punjab, Haryana, Rajasthan, Gujarat and the peninsular India[5]. *Costus speciosus* rhizomes are rich in ascorbic acid, beta-carotene, alpha – tocopherol, glutathione, phenols, flavanoids, alkaloids, terpenoids, steroids, tannins, phenolics, red sugar, Eremanthin[6]. It also contains diosgenin, prosapogenin B of dioscin, dioginone, cycloartanol, 25-en-cycloartenol and octacosanoic acid[7]. Action of *Costus speciosus* are astringent, purgative, depurative, anti-inflammatory, anthelmintic, antivermin, maggoticide, antifungal[8]. The extract of different part of

**Author for Correspondence*

Costus speciosus have been reported to possess anti-inflammatory, antioxidant and anti-nociceptive activity.
Therefore, this research is undertaken to proof that *Costus speciosus* rhizome extract can be used for treatment of arthritis because it is able to reduces oxidative stress and hence, DNA damage. Anti-arthritic activity was evaluated using *in-vitro* techniques.

MATERIALS AND METHODS

Drugs and chemicals

Indomethacin (gift sample) was received from SCOAT Pharma pvt. Ltd, Hyderabad, Andhra Pradesh. Freund's complete adjuvant was purchased from Sigma Aldrich. Rest of the chemicals was purchased from CDH, pvt. Ltd, New Delhi.

Plant collection and authentication

Semi-dried rhizomes of *Costus speciosus* was collected and authenticated in month of September from VHCA herbals, Karnal, Haryana.

Extraction of rhizome of Costus speciosus

The dried powder of *Costus speciosus* koen rhizome was continuously extracted using soxhlet apparatus with chloroform for 60 min to remove the wax, oil, chlorophyll, and non-polar phenolic compounds. Then, the residue was extracted with 80% methanol solution for 12 hrs at temperature 70 – 75 º C. The extract was concentrated under vacuum using rotary vacuum evaporator, dried and weighed[9]. Phytochemical estimation of extract was done[10-12].

Estimation of flavonoid content

About 1.0g of sample was extracted repeatedly with 100ml of 80% methanol. The whole solution was then filtered through Whatmann No.42 filtered paper. The filtrate was transferred into a crucible and evaporated to dryness on a water bath, and weighed. The percentage flavonoid content was then calculated[13].

Animals

Animals were procured from Central Animal House, MIET, Meerut. Animals were approved by Institutional Animal Ethic Committee (IAEC) of MIET, Meerut. Approval number CPCSEA No. 711/02/a/CPCSEA was given for this work.

Swiss albino mice (20–25 g) and Wistar albino rats (150-200) were obtained from the animal house of the Deptt. Of Pharmaceutical technology, Meerut Institute of Engineering and Technology, Meerut (U.P), India. They were acclimatized in the laboratory for seven days before the experiments. The temperature of the experimental animal room was maintained to be 22ºC (±3ºC). Relative humidity was maintained between 50–60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. Animals of same group were caged together. Research was conducted in accordance with the Institutional Animal Ethics Committee of MIET.

Arthritis induction by FCA administration

0.1ml of Freund's complete adjuvant (FCA) (composed of 1 mg/ml heat killed Mycobacterium tuberculosis, mineral oil and mannide monooleate) was injected in sub-plantar region of left hind paw of rats.

Treatment protocol

Rats were divided into seven groups. Group I served as control group and received 0.5% of CMC. Group II served as arthritic control and received 0.5% CMC and FCA. Group III served as standard group and received Indomethacin (10 mg/ml, p.o). Group IV and group V served as test group and received prophylactic low dose of test extract (PLDE) (100 mg/kg) and prophylactic high dose of test extract (PHDE)(200 mg/kg), respectively. Group VI and group VII served as test group and received therapeutic low dose of test extract (TLDE) (100 mg/kg) and therapeutic high dose of test extract (THDE) (200 mg/kg). All the group except group I, received 0.1 ml of FCA along with treatments. All the treatments of group I to V were started at day 0 and continued till day 28. Treatments of group VI and VII were started at day 04 and continued till day 28.

Nitric Oxide estimation

About 1 mL of blood was drawn from the animals of each group by retro orbital puncture to estimate the serum nitric oxide levels. The blood samples were centrifuged at 3000 rpm for about 10 min to collect the serum, followed by biochemical estimation of nitric oxide. Sample solutions were taken in test tubes and treated with Griess reagent (1%sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride and 2.5% hydrochloric acid). The colorimetric reaction was allowed to proceed for 10 min at room temperature, and optical density was measured at 550 nm using a spectrophotometer. The concentrations of nitrite were calculated from a standard curve established with serial dilutions of sodium nitrite[14].

Estimation of Biochemical Parameters

Arthritic joint tissue preparation

Rats were euthanized by cervical dislocation carefully. Tissues from arthritic joint were removed. 1 gm of arthritic joint tissue was taken and homogenized with 10 times (w/v) by homogenizer in ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from arthritic joint tissue were separated and used to determine protein, lipid peroxidation and glutathione. Protein concentration was determined using purified bovine serum albumin as standard[15].

Estimation of glutathione (GSH) level in arthritic joint tissue homogenate

Procedure

The equal quantity of arthritic joint tissue homogenate (w/v) and 10% TCA was mixed and centrifuged at 3000 rpm for 15 min to separate the proteins. To 0.01 ml of this supernatant, 2 ml phosphate buffer (pH 7.4), 0.5 ml 5, 5′ dithiobisnitro benzoic acid (DTNB) and 0.4 ml double distilled water was added. The mixture was vortexed and the absorbance was read at 412 nm within 15 min. Measurement of reduced glutathione (GSH) value was expressed as µ moles GSH/mg protein[16].

Determination of Lipid Peroxidation (Measurement of MDA/TBARS) level

Procedure:

1 ml. of suspension medium was taken from the 10% tissue homogenate in a tube. 0.5 ml. of Trichloroacetic acid (TCA) was added to it, followed by 0.5 ml. of 8% Thiobarbituric acid (TBA) reagent. The tubes was covered

with aluminum foil and kept in shaking water bath for 30 min. at 80^0C. After 30 min. tube was taken out and kept in ice cold water for 30 min. These were centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm, at room temperature against appropriate blank (1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA.). MDA value was expressed as n moles MDA/mg of protein[17,18].

DNA estimation

For extraction of nucleic acid 5 ml of 0.6 N ice cold of perchloric acid (PCA) was added to 1 ml of arthritic joint tissue homogenate of each rats of each group and tubes were kept in the refrigerator for 10-15 min. They were centrifuged for 5 min at 4000 rpm. The supernatant was discarded. The pellets were dissolved in 4ml of 0.3N NaOH solution and were incubated in water bath at temperature of 38±1ºC for 1 hour. During this time the tubes were shaken several times to thoroughly dissolve the pellet. After 1 hour the tubes were removed from the incubation bath and 4 ml of hydrolysate formed was separated. 2 ml of hydrolysate was utilized to determine DNA content. Indole solution (0.04% aqueous) was prepared by dissolving 40 mg of indole in 10 ml water. From this the required amount of indole solution was taken and mixed with exactly the same volume of concentrated HCl. 2ml of this HCl: Indole (1:1) solution was pippetted into 2 ml of NaOH hydrolysate. The tubes were then placed in boiling water bath for 10 min. Tubes were cooled and a pink coloration was obtained. To this was added 4 ml of $CHCl_3$. The tubes were shaken vigorously. After which, two layers were obtained, an upper aqueous layer and lower $CHCl_3$ layer. The upper aqueous layer was removed and another 4 ml of $CHCl_3$ was added to this. Shaking tubes were centrifuged to obtain two layers. The upper aqueous layer which was now clear of all precipitate was removed and optical density was read at 440 nm[19].

In-vitro anti-arthritic activity:

Inhibition of protein denaturation.

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Costus speciosus* test extracts (*CSTE*) (100 and 200 mcg/ml of final volume). pH was adjusted to 6.3 using a small amount of 1 N HCl. The samples were incubated at 37ºC for 20 min and then heated at 57ºC for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin[21]. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{P. I} = 100 - \frac{(\text{O.D.of T} - \text{O.D.of P.C})}{\text{O.D of control}} \times 100$$

T = test

P.C = product control

The control represents 100% protein denaturation. The results were compared with indomethacin (200 mcg/ml) treated samples.

Effect on Membrane Stabilization

Preparation of red blood cell suspension.

Fresh rat blood was collected into centrifuge tube containing 200mM 0.5 ml EDTA. The tubes were centrifuged at 3000 rpm for 15 minutes and washed thrice with equal volume of normal saline. The volume of RBC was measured and reconstituted as 10% v/v suspension with normal saline[22].

Hypotonic solution induced hemolysis.

The reaction mixtures (4.5 ml) consisted of 2 ml hypotonic saline (0.25% NaCl), 1 ml 0.15 M phosphate buffer (pH 7.4) and 1 ml test solution (100 and 200 mcg/ml of final volume) in normal saline. 0.5 ml of 10% rat RBC in normal saline was added. For control tests, 1 ml of isotonic saline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56ºC for 30 minutes. The tubes were cooled under running tap water for 20 minutes. The mixtures were centrifuged and the optical density (O.D) of the supernatants read at 560 nm[23]. The Percent inhibition was calculated.

The control represents 100% lysis. The results were compared with indomethacin (200 mcg/ml) treated samples.

Proteinase inhibitory action

The reaction mixtures contained 0.06 mg/ml trypsin, 1.0 ml. 25 mM tris-HCl buffer (pH 7.4) and 1.0 ml aqueous solution of *Costus speciosus* (100 and 200 mcg/ml of final volume). The mixtures were incubated at 37ºC for 5 minutes. Then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated[24]

RESULTS

Phytochemical Screening

Phytochemical screening revealed the presence of flavonoids, saponins glycoside, and carbohydrate

Quantitative estimation of flavonoids

Percent flavonoid content was found to be 8%

Nitric oxide determination

The result has shown that there is significant increase in level of nitric oxide in arthritic control group. It can be seen in table1 and figure1 that there is significant reduction in level of nitric oxide in standard indomethacin group, PHDE (200 mg/kg), PLDE (100 mg/kg), and THDE (200 mg/kg) 2.896 ± 0.022 µg/ml, 2.868 ± 0.014 µg/ml, 3.231 ± 0.013 µg/ml and 3.213 ± 0.022 µg/ml respectively. However, the reduction of nitric oxide level in TLDE (100 mg/kg) is less significant.

MDA level

Results demonstrate that in arthritic control group there is increase in MDA level upto 5.00±0.23 nm/mg of protein. Treatment of various group significantly lowered MDA level to 1.42±0.11 nm/mg of protein in standard group, 3.11±0.18 nm/mg of protein in PLDE, 1.76±0.16 nm/mg of protein in PHDE and 2.36±0.08 nm/mg of protein in THDE. However, in TLDE the lowering of MDA level is less significant i.e. 4.07±0.18 nm/mg of protein (Table 2, Figure 2).

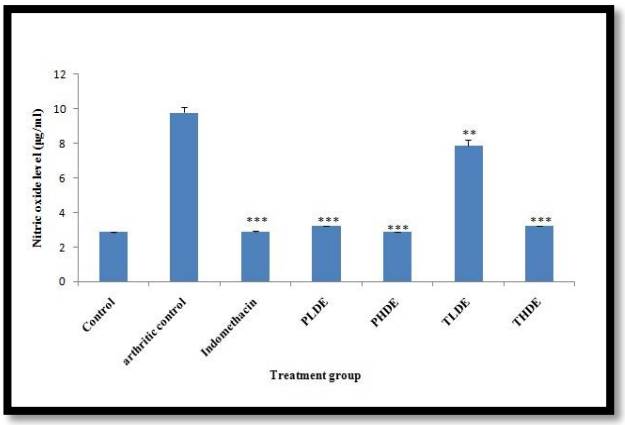

Fig. 1: Effect of *Costus speciosus* Koen extract on Nitric oxide level (µg/ml) of experimental animals of different groups at $p<0.05$.

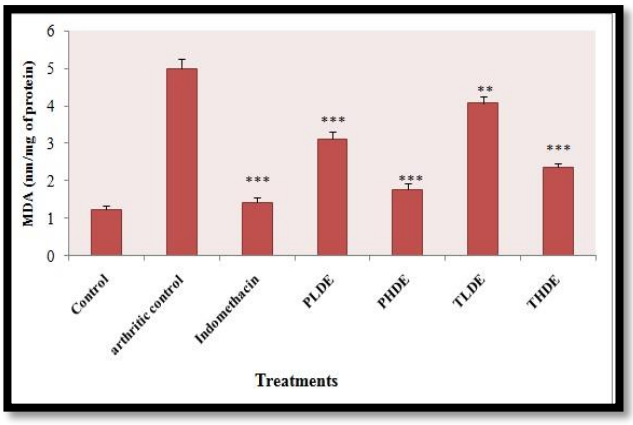

Fig. 2: Effect of Costus speciosus Koen extract on MDA level (nm/mg of protein) of experimental animals of different group at $p<0.05$

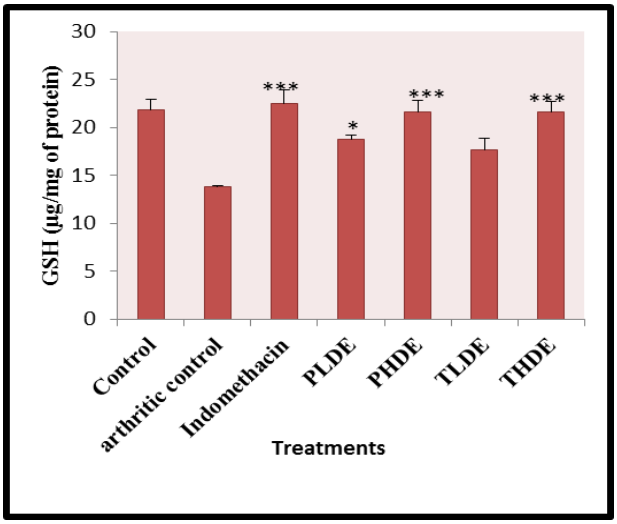

Fig. 3: Effect of *Costus speciosus* Koen extract on GSH (µg/mg of protein) level of experimental animals of different groups at $p<0.05$.

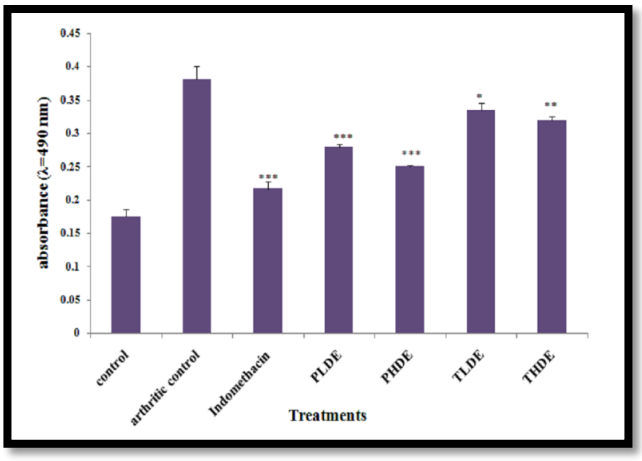

Fig. 4: Effect of Costus speciosus Koen extract on DNA damage evaluated by absorbance at λ=490 nm experimental animals of different groups at $p<0.05$.

Table 1: Nitric oxide level (µg/ml) of different group at $p<0.05$. All values are expressed in Mean±SEM.

Treatment groups	Nitric oxide level (µg/ml)
Control	2.848±0.025
Arthritic control	9.786 ± 0.304
Indomethacin(10mg/kg)	2.896 ± 0.022***
PLDE (100 mg/kg)	3.231 ± 0.013***
PHDE (200 mg/kg)	2.868 ± 0.014***
TLDE (100 mg/kg)	7.840 ± 0.378**
THDE (200 mg/kg)	3.213 ± 0.022***

GSH level

Results showed that in arthritic control group there is significant decrease in GSH level to 13.83±0.67 µg/mg of protein. Administration of drugs to different treatment groups increased GSH level. The increase is highly significant in standard group, PHDE and THDE to 22.53 ± 1.35 µg/mg of protein, 21.61 ± 1.17 µg/mg of protein and 21.61 ±1.08 µg/mg of protein respectively. Increase if GSH level is less significant in TLDE i.e. 18.33±0.05 µg/mg of protein (Table 3, Figure 3).

DNA estimation

Referring to data showed in table and figure , it was seen that there was increases in absorbance in arthritic control group and there was significant decrease in absorbance in standard group (Indomethacin), PLDE (100 mg/kg) and PHDE (200 mg/kg) at $p<0.05$. In TLDE (100 mg/kg) and THDE (200 mg/kg) decrease in absorbance is less significant. On the basis of absorbance, percent inhibition of DNA damage was calculated, Standard (Indomethacin) afford maximum protection of 43.04% against DNA damage, followed by PHDE (200 mg/kg) 34.38%, PLDE (100 mg/kg) 26.50%, THDE (200 mg/kg) 16.25% and TLDE (100 mg/kg) 12.07% (Table 4, Figure 4).

In-vitro anti-arthritic activity

In-vitro anti-arthritic activity was performed using inhibition of protein denaturation activity, effect on membrane stabilization activity and proteinase inhibitory activity. Anti-arthritic activity of *CSTE* (low dose and high dose) was compared with single dose of Indomethacin.

Inhibition of protein denaturation

Table2 : Effect of Costus speciosus Koen extract on MDA level (nm/mg of protein) of experimental animals of different group at p<0.05. All values are expressed in Mean ± SEM

Treatments	MDA (nm/mg of protein)
Control (0.5 % CMC)	1.22 ± 0.09
Arthritic control	5.00 ± 0.23
Indomethacin (10 mg/kg)	1.42 ± 0.11***
PLDE (100 mg/kg)	3.11 ± 0.18***
PHDE (200 mg/kg)	1.75 ± 0.16***
TLDE (100 mg/kg)	4.07 ± 0.18**
THDE (200 mg/kg)	2.36 ± 0.08***

Table 3: Effect of Costus speciosus Koen extract on GSH (µg/mg of protein) level of experimental animals of different groups at p<0.05.

Treatments	GSH (µg/mg of protein)
Control (0.5 % CMC)	21.82 ± 1.119
Arthritic control	13.83 ± 0.67
Indomethacin (10 mg/kg)	22.53 ± 1.35***
PLDE (100 mg/kg)	18.33 ± 0.05*
PHDE (200 mg/kg)	21.61 ± 1.17***
TLDE (100 mg/kg)	17.70 ± 1.10
THDE (200 mg/kg)	21.61 ± 1.08***

In inhibition of protein denaturation, *CSTE* at dose 100 mcg/ml and 200 mcg/ml showed percent inhibition of 46.145% and 64.54% respectively. Percent inhibition by *CSTE* (200 mcg/ml) is significantly higher (p<0.05) than that of Indomethacin (42.21%).

Percent inhibition of *CSTE* at dose 100 mcg/ml is almost similar to Indomethacin (200mcg/ml) (Table 5).

Membrane stabilizing activity

In membrane stabilization activity, *CSTE* at dose 100 mcg/ml and 200 mcg/ml showed percent inhibition of 43.17% and 74.07% respectively but lower than Indomethacin (94.18%) which showed highly significant membrane stabilization (p<0.05) (Table 5).

Proteinase inhibitory activity

In Proteinase inhibitory activity, *CSTE* at dose 100mcg/ml and 200mcg/ml showed percent inhibition of 47.54% and 66.96% respectively. Indomethacin (88.16%) which showed highly significant proteinase inhibitory activity (p<0.05). *CSTE* (200 mcg/ml) can be considered to have significant Proteinase inhibitory activity (Table 5).

DISCUSSION

In the present study, we have obtained methanolic extract of rhizome of *Costus speciosus* using soxhlet apparatus and determined flavonoid content. The effect of methanolic extract was evaluated on oxidative stress parameters such as GSH, MDA and nitric oxide. A research showed that lipid peroxidation-derived DNA adducts, highly accumulated in whole blood cells of RA. DNA damages play a significant role in the development of RA and/or its complications[25]. Therefore, DNA level determination was done, since oxidative stress from oxidative metabolism causes base damage, as well as

Table4: Effect of Costus speciosus Koen extract Percent inhibition of DNA damage in experimental animals of different group at p<0.05.

Treatment	Mean ± SEM absorbance at 490 nm	Percent inhibition of DNA damage
Control (0.5% CMC)	0.175±0.011	54.06 %
Arthritic control	0.381±0.019	-
Indomethacin (10 mg/kg)	0.217±0.011***	43.04%
PLDE (100 mg/kg)	0.280±0.004***	26.50%
PHDE (200 mg/kg)	0.250±0.002***	34.38%
TLDE (100 mg/kg)	0.335± 0.010*	12.07%
THDE (200 mg/kg)	0.319±0.006**	16.25%

strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated, e.g. $O_2^{\bullet -}$ (superoxide radical), $OH^{\bullet}$ (hydroxyl radical) and H_2O_2 (hydrogen peroxide)[26].

Intra-articular injection of FCA produced inflammation of the joint with a peak effect occurring on day 4 where a maximum increase in the levels of myeloperoxidase and inflammatory mediators like PGE2, TNF-α, and nitric oxide was observed. This was associated with oxidative stress with a marked reduction in the levels of glutathione and an increase in the lipid peroxidation as indicated by the higher levels of thiobarbituric acid reactive substances (TBARSs)[27].

The role of NO has been well established in an inflammatory response. Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids to form radical intermediates that bring about cellular damage. MDA, a major end product of this reaction, is an index of lipid peroxidation and has been estimated as TBARS. In our study, standard drug and all the treatment group brought down the level of NO and MDA. Beside this, the level of glutathione that traps free radicals and prevents oxidative stress is also decreased in arthritis. All the treatment group elevated of GSH level. Concentration of DNA and percent inhibition of DNA damage was also evaluated. Standard as well as all treatment groups significantly worked against DNA damage. So, a hypothesis can be laid down that since methanolic extract of *Costus speciosus* rhizome act against oxidative stress, it has the capacity to prevent DNA damage.

Denaturation of protein is a kind of cellular response. Suggestions have been made that protein denaturation and macroglobulin formation cause the protein to become auto-antigenic, thus initiating the immune response and producing biochemical changes in connective tissue which ultimately leads to rheumatoid arthritis[20]. Production of auto antigen in certain arthritic disease may due to denaturation of protein. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. *CSTE* (100 mcg/ml

Table5: Percent inhibition of different in-vitro anti-arthritic model of Indomethacin (200 mcg/ml), CSTE (100mcg/ml), CSTE (200mcg/ml) at $p<0.05$.

Treatment	Protein denaturation (%)	Membrane stabilization (%)	Proteinase inhibition (%)
Indomethacin (200 mcg/ml)	42.29± 3.69	94.18± 1.30	88.76± 2.72
Costus speciosus test extract (100 mcg/ml)	46.14±2.32	43.17±2.41***	47.541± 5.009***
Costus speciosus test extract (200 mcg/ml)	64.54±2.10***	74.04 ± 2.11**	66.96±1.56***

and 200 mcg/ml) is capable of preventing denaturation of protein[28].

Exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium, heat, methyl salicylate or phenylhydrazine results in the lysis of the membranes, accompanied by haemolysis and oxidation of hemoglobin. Since, red blood cell (RBC) membranes are similar to lysosomal membrane Components, the inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators[29]. *CSTE* (100 mcg/ml and 200 mcg/ml) stabilized the red blood cell membrane.

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of Proteinase which carries in their lysosomal granules many neutral serine proteinases. Leukocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinases inhibitors[30].

REFERENCES

1. Boon NA, Colledge NR, Walker Brian. Davidson's principle and practice of medicine. Churchill livingtone. Elsevier;2006
2. Soeken KL, Miller SA, Ernst E: Herbal medicines for the treatment of rheumatoid arthritis: a systematic review. Rheumatology 2003; 42(5):652-659.
3. Bhangle J, Acharya S: Antiarthritic activity of *Cynodon dactylon* (L.) Pers. Indian Journal of Experimental Biology 2014; 52:215-222.
4. Altindag O, Karakoc M, Kochigit A, Celik H, Soran N. Increased DNA damage and oxidative stress in patient with RA. Clinical Biochemistry 2007; 40(3-4).
5. Srivastava S, Singh P, Mishra G, Jha KK., Khosa RL. *Costus speciosus* (Keukand): A review. Der Pharmacia Sinica 2011; 2 (1):118-128.
6. Qiao CF, Li QW, Dong H, Xu LS, Wang ZT. Studies on chemical constituents of two plant from *Costus.* Zhonqque zhong Yao Za Zhi 2002; 24(2):123-125
7. Vijayalakshmi M.A, Sarada N.C. Screening of *Costus speciosus* extracts for anti-oxidant activity. Fitoterapia 2008; 79(3):197-198.
8. Khare CP. Indian Medicinal Plant. Newyork: Springer-Verlag Berlin Heidelberg ;2007.
9. Chang YQ, Tan SN, Yong JWH, Ge L. Determination of flavonoids in costus speciosus and etlingera elatior by liquid chromatography-mass spectrometry. Analytical Letters 2012; 45: 345–355.
10. Trease GE, Evans WC, 2002. A Text book of Pharmacognosy. 15th ed. Harcourt brace & Co. Asia, Pvt. Ltd., W.B. Saunders Company Ltd.
11. Khandelwal KR., 2010. Practical Pharmacognosy. India, Nirali Prakashan.
12. Al-Mailiki ADM. Isolation and Identification of Phenolic Compounds from Elettaria cardamomum Seeds and Study of their Medicinal Activity against Pathogenic Bacteria of Prostate Gland. Journal of Misan Researches 2011; 8(15): 13-35
13. Obadoni BO, Ochuka PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure Applied Sci 2001; 8:203-208.
14. Menaka KB, Ramesh A, Thomas B, Kumari NS. Estimation of nitric oxide as aninflammatory marker in periodontitis. Journal of Indian Society of Periodontology 2009; 13(2).
15. Lowery OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. Journal of Biological Chemistry 1951; 193:265-275.
16. Ellman GI: Tissue sulphydryl group. Archives of Biochemistry and Biophysics. 1959;82:70-77
17. Mesa MD, Aguilera CM, Gil A. Experimental model of oxidative stress in cardiovascular diseases and diabetes. Basu S, Wiklund L editors. Studies on Experimental Models. A product of Humana press; 2011.p. 142.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by Thiobarbituric acid reaction. Analytical Biochemistry 1979; 95:351-358.
19. Malik K, Awan K, Lone KP. Effect of feeding rapeseed meal on the nucleic acids concentrations in liver of Japanese quail. *Afr. J* Microbiological Research 2012; 6(18): 3984-3988
20. Jayaprakasam R, Ravi TK. Evaluation of anti arthritic activity of the root extract of *Acalypha indica* linn. using *in vitro* techniques. Int J of Phyto Pharm 2012; 2(6):169-173.
21. Deshpande V, Jadhav VM, Kadam VJ. In-vitro anti-arthritic activity of *Abutilon indicum* (Linn.) Sweet. Journal of Pharmacy Research 2009; 2(4):644-645
22. Gupta SK, Gupta A, Pakash D, Vedpal. In – vitro antiarthritic activity of Ethanolic extract of *Callicarpa macrophylla*. International research Journal of pharmacy 2013; 4(3): 160-162.

23. Sakat SS, Juvekar AR, Gambhire MN. In vitro antioxidant and anti inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(1): 146-155
24. Kumar VL, Roy S. *Calotropis procera* latex extract affords protection against inflammation and oxidative stress in freund's complete adjuvant-induced monoarthritis in rats. Mediators of Inflammation 2007; 1-7.
25. Ogawa M, Matsuda T, Tanaka T. DNA damage in RA. An age dependant increase in the lipid peroxidation derived DNA adduct, Heptanone-Etheno-2′-Deoxycytidine. Autoimmune diseases. 2013; 1-10.
26. Slupphaug G, Kavli B, Krokan HE. The interacting pathways for prevention and repair of oxidative DNA damage. Mutation Research 2003; 531(1-2):231-51
27. Lemke TL, Williams DA, Roche VF, Zito SW. Foye's principle of medicinal chemistry. New Delhi: Wolters Kluwer pvt. Ltd; 2008.
28. Sangeetha M, Kousalya K, Lavanya R, Sowmya C, Chamundeeswari D, Reddy UM. *In-vitro* Anti-inflammatory and Anti-arthritic Activity of Leaves of *Cleodendron Inerme.* Research Journal of Pharmaceutical, Biological and Chemical Sciences 2011; 2(1): 822.
29. Anosike CA, Obidoa O, Ezeanyika LUS. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg *(Solanum aethiopicum)* DARU Journal of Pharmaceutical Sciences. 2012; 20(76) :2-7
30. Mishra NK, Bstia S, Mishra G, Chowdary KA, Patra S: Anti-arthritic activity of *Glycyrrhiza glabra, Boswellia serrata* and their synergistic activity in combined formulation studied in Freund's adjuvant induced arthritic rats. Journal of Pharmaceutical Education and Research 2011; 2(2): 92-98.