

Methanolic Extract of *Curcuma caesia* Roxb. Prevents the Toxicity Caused by Cyclophosphamide to Bone Marrow Cells, Liver and Kidney of Mice

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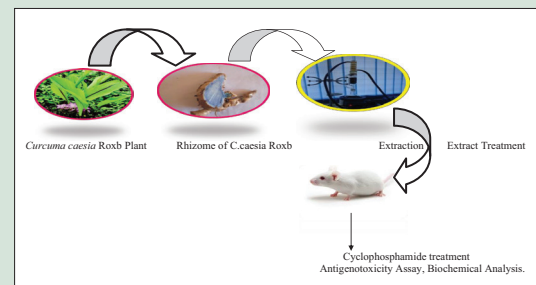
ABSTRACT

Introduction: With an ever increasing cause of cancer, it has been recommended to treat with conventional drugs, however because of the side effects caused by the conventional drugs, the research on medicinal plants has been intensified due to their less adverse and toxic effects. **Objectives:** The primary objective of the present study was to evaluate the protective effect of the medicinal plant *Curcuma caesia* Roxb. against free radicals $ABTS^+$ and O_2^- . Also it was aimed to evaluate the protective effect of *C. caesia* Roxb. against the chemotherapeutic drug Cyclophosphamide and its side effects in liver and kidney. **Methods:** The rhizomes of the plant was extracted with methanol through soxhlet and its antioxidant activity was tested against $ABTS^+$ and O_2^- . For antigenotoxic studies, animals were divided into eight groups and micronucleus assay was employed and for biochemical analysis serum sample was collected from the blood and SGOT, SGPT analysis was performed. Also the biochemical analysis was performed from both the liver and kidney. **Results:** The methanolic extract of *Curcuma caesia* Roxb. was found to scavenge the free radicals $ABTS^+$ and O_2^- . the micronuclei formation was found to be increased in the positive control group as compared to the negative control group significantly ($P < 0.002$) however increase in the number of micronuclei was found to be decrease with the pretreatment of the extract at different concentrations significantly as compared to the negative control groups ($P < 0.01$, $P < 0.005$, $P < 0.001$). The increased level of serum SGPT and SGOT as well as peroxidation level in both liver and kidney due to treatment of cyclophosphamide was also found to be decreased with the pretreatment of the extract significantly as compared to the positive control groups. There was decreased in the level of endogenous antioxidant such as GSH and GR in the positive control group however decreased level of GSH and GR was found to be increased with the pretreatment of the methanolic extract of *C. caesia* Roxb. **Conclusion:** The present study suggested that the methanolic extract of *C. caesia* Roxb has not shown any genotoxicity and reduces the genotoxicity caused by cyclophosphamide. It was also to have the protective effects against the liver and kidney. So it could be provided as one of the herbal supplementation in chemoprevention of CP to ameliorate the side effects of it.

Key words: 2,2' azino bis (ethylbenzthiazolone-6-sulfonic acid) radical cation assay, Superoxide dismutase assay, Cyclophosphamide, micronucleus assay, *Curcuma caesia* Roxb., Oxidative stress

SUMMARY

• Cancer is characterized by uncontrolled growth of cells and much research has been done for the past several years from various disciplines for the treatment of cancer but till now no therapy has been discovered. Treatment of cancer with chemotherapeutic drugs has been suggested to prevent cancer cells however they are often limited with their toxicity to normal cells. Therefore it has been suggested that the supplementation of medicinal plants which are rich source of antioxidants can decrease the toxic effect caused by chemotherapeutic drugs. *Curcuma caesia* Roxb is a medicinal plant which has high antioxidant activity, as per present study, methanolic extract of *Curcuma caesia* Roxb prevents the toxicity caused by cyclophosphamide (chemotherapeutic drug) in bone marrow cells by reducing the micronuclei formation; it also prevents the hepatotoxicity and nephrotoxicity caused by cyclophosphamide, so it can be used as a supplement in cancer treatment with cyclophosphamide.



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INTRODUCTION

With an ever increasing cause of cancer due to diet, environment, and carcinogenic virus^[1,2] conventional drugs have been recommended for cancer patients. Such treatments lengthened the life or may permanently cure, but most treatments, experiences side effects such as miserable pain, blood clots fatigue, and infection.^[3] However, due to less toxic and adverse effects, the research on medicinal plants or herbs has been intensified,^[4] since they have profound active ingredients yielding important breakthrough in cancer prevention and treatment and they have been using as a first line of cancer-fighting agent in developing countries.^[5]

Cyclophosphamide (CP) is a nitrogen mustard alkylating agent^[6] used in various types of cancer chemotherapy, but the International Agency for

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Research Centre has identified it as a carcinogen for both animals and humans.^[7] CP therapy causes injuries to normal tissue,^[8] and peroxidative damage to kidney and other vital organs in which such side effects are supported by the reactive oxygen species (ROS)^[9] such as acrolein (ROS) and phosphoramidate mustard (ROS)^[10] produced by the metabolic activation of CP in the liver by cytochrome P4₅₀ mix functional oxidase system.^[11,12] *Curcuma caesia* Roxb. (black turmeric) is a perennial herb with bluish black rhizomes and one of the endangered species amongst the medicinal plants found in Manipur.^[13] Many of the researches worked on it like anti-fungal activity,^[14] smooth muscle relaxant and anti-asthmatic activity,^[15] bronchodilating activity,^[16] antioxidant activity,^[17] anxiolytic and central nervous system depressant activity, locomotor depressant, anti-convulsant,^[18] anthelmintic activity,^[19] anti-bacterial activity,^[20] and anti-ulcer activity.^[21] Methanolic extract of the rhizome of *Curcuma caesia* Roxb. (MECC) has high phenol content,^[22] it is also a good source of antioxidant and antimutagenic activity.^[23] The rhizomes of the plant are also a rich source of many phytoconstituents such as essential oils with camphor, ar-turmerone, (Z) ocemene, ar-curcumene, 1,8-cineole, elemene, borneol, bornyl acetate, and curcumene etc.^[24] Till now, no data are available on the antigenotoxic activity of the rhizome of MECC against CP, so the present study was undertaken to investigate the prevention of toxicity by the rhizome of MECC caused by CP in bone marrow cells and oxidative stress produced in the liver and kidney.

MATERIALS AND METHODS

Plant material collection and extraction

Rhizomes of *C. caesia* Roxb. were collected from the region of Nambol, Bishnupur District, Manipur, India and were cut into pieces and sun-dried. The dried rhizomes were coarsely powdered and extracted with methanol through soxhlet at a temperature of 50–60°C for a period of 12–24 h. The crude extract was dried in a water bath and kept for further uses.

Preliminary phytochemical screening

Qualitative preliminary phytochemical screening was performed following.^[25]

Drugs and chemicals

All the drugs and chemicals used in this experiment were procured from Segma sales, Silchar, Assam, India, which were kindly provided by Himedia, India.

2,2' azino bis (ethylbenzthiazolene-6-sulfonic acid) radical cation decolourisation assay

The assay was performed following^[26] with slight modifications. ABTS radical cation was generated by the addition of 7 mM ABTS and 2.45 mM potassium persulphate. The reaction mixtures were allowed to stand for 12–16 h at 30°C in the dark. After 16 h, the reaction mixture was diluted with ethanol or phosphate buffer saline (pH = 7.4). Following that 0.3 mL of ABTS⁺ and 0.5 mL of extract solution (5–100 µg/mL) were mixed and absorbance was read at 734 nm without any incubation period against the sample blank prepared by mixing 0.3 mL of methanol and 0.5 mL of Dimethyl sulfoxide (DMSO). Similarly, control was also read in the same wavelength by adding together of 0.3 mL of 2,2' azino bis (ethylbenzthiazolene-6-sulfonic acid) radical cation, 0.5 mL of DMSO and 1 mL of methanol. With the same procedure measurement of the gallic acid standard was also recorded. Percentage of inhibition was calculated using the formula given below:

$$(\%) \text{ inhibition} = \frac{Ab - Aa}{Ab} \times 100$$

Where Ab is the absorption of the control and Aa is the absorption of the extract sample.

Superoxide dismutase assay

It was performed following^[27] with slight modifications. The reaction mixture contain 1 mL of different concentrations of extract (20–200 µg), 1 mL of 156 µM nicotinamide adenine dinucleotide hydrogen (NADH), 1 mL of 60 µM nitroblue tetrazolium, and 1 mL of 468 tetrazolium(NBT), and 1 mL of 468 µM phenazine methosulphate(PMS) in phosphate buffer (pH = 8.3). The reaction mixture was incubated at 25°C for 10 min and absorbance was taken against blank at 560 nm. The standard taken was gallic acid. The inhibition mixture was calculated using the formula:

$$(\%) \text{ inhibition} = \frac{Ab - Aa}{Ab} \times 100$$

Where, Ab is the absorption of the control and Aa is the absorption of the extract sample.

Experimental design

The animals were kindly procured from the Pasteur Institute, Shillong, India and were acclimatized for 15 days. The study was conducted on 25–30 g body weight male Swiss albino mice. They were maintained under controlled conditions of temperature and light (12 h light: 12 h dark). They were provided standard mice feed. The study protocol was approved by the Institutional Ethical Committee (IEC/AUS/2-013-33, dt. 20/3/13 Assam University, Silchar, India).

The experimental animals were divided into eight groups each containing five mice designated as follows:

- Group 1: Negative control (NC): Each animal received distilled water
- Group 2: Positive control (PC): CP was administered intraperitoneally at a dose of 50 mg/kg, b.wt
- Group 3: Animals received 100 mg/kg, b.wt of MECC only intraperitoneally
- Group 4: Animals received 250 mg/kg, b.wt of MECC only intraperitoneally
- Group 5: Animals received 500 mg/kg, b.wt of MECC only intraperitoneally
- Group 6: Pretreatment: MECC was administered at a dose of 100 mg/kg, b.wt (i.p) followed by CP (i.p) treatment 2 h later
- Group 7: Pretreatment: MECC was administered at a dose of 250 mg/kg b. wt (i.p) followed by CP (i.p) treatment 2 h later
- Group 8: Pretreatment: MECC was administered at a dose of 500 mg/kg b. wt (i.p) followed by CP (i.p) treatment 2 h later.

After 7 days of the experimental period, the animals were sacrificed, and parameters described below were studied.

Serum sample collection

The blood sample was collected from the heart and kept it undisturbed for 2 h. Serum was then removed by centrifugation at 10,000 g for 10 min and isolated serum sample was kept in –80°C for further analysis of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Besides, the removed kidney and liver were washed with phosphate buffer saline and blotted with filter paper and kept in the deep freezer for further analysis.

Micronucleus assay

Mice bone marrow micronucleus test was carried out according to.^[28] Bone marrow cells from both the femurs of each animal were flushed out with fetal bovine serum albumin (FBS) in a centrifuge tube. The cell suspensions were centrifuged at 10,000 rpm for 10 min and supernatant was removed. The pellet was resuspended in FBS before being used for preparing slides. The air-dried slides were stained with May Grunwald stain and Geimsa stain. Thousand polychromatic erythrocytes (PCEs) were scored for each group of animals to determine the frequency of

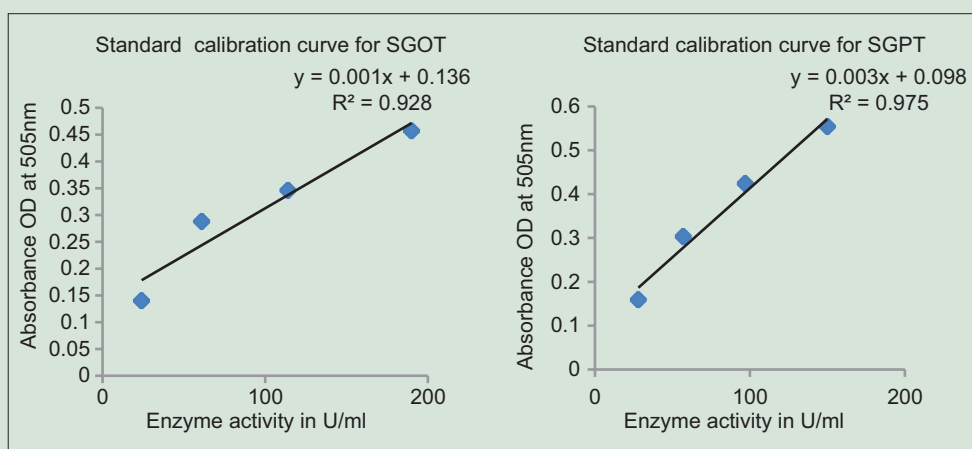


Figure 1: Standard calibration curve for serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase

micronucleated polychromatic erythrocytes. All the slides were coded and scored by the same observer. The percentage reduction in the frequency of micronuclei was calculated using the formula given by^[29]:

$$\text{Reduction (\%)} = \frac{\text{mean DI in A} - \text{mean DI in B}}{\text{mean DI in A} - \text{mean DI in C}}$$

A = Group treated with CP

B = Group treated with CP plus methanol extract of the rhizome

C = Negative groups

DI = Damage index

Biochemical assays

Determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase

It was performed according to the protocol provided by^[30] in a commercial kit (SGOT [ASAT] and SGPT [ALT] kits). Each enzyme activity (U/mL) was calculated from the standard curves generated [Figure 1].

Quantitative assay for lipid peroxidation

The assay was performed following^[31] with slight modifications. 0.2 g of the sample was homogenized in 2 mL of 0.2 M KCl followed by centrifugation at 10,000 rpm for 10 min in cooling centrifuge (Heraeus Biofuge Startos centrifuge). 0.5 mL of the homogenate was mixed with 100 μ L of 10 mM FeCl₃ and incubated at 37°C for 30 min. After incubation 400 μ L of TCA, 50 μ L of BHT, 0.5 mL of TBA, and 50 μ L of 0.25N HCl were added and heated at 100°C for 60 min. The reaction mixtures were cooled and then centrifuged it. Absorbance was recorded against blank. The percentage inhibition was calculated using the formula given below:

$$(\%) \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of glutathione reduced level

Glutathione reduced (GSH) was estimated following^[32] with slight modifications. 0.1 g of each sample was homogenized in 2.5 mL of 10% TCA followed by the centrifugation at 10,000 rpm for 10 min. 0.1 mL of the supernatant was mixed with 0.9 mL of 0.2 M phosphate buffer and 0.2 mL of 0.6 mM (5,5'-dithiobis-[2-nitrobenzoic acid]) (DTNB) and the absorbance was read at 412 nm against blank. The level of GSH was expressed as nmole of GSH/g tissue.

Estimation of cytosolic glutathione reductase

Glutathione reductase (GR) was assayed in the liver and kidney following^[33]. 0.1 g of tissue was homogenized in 1 mL of phosphate buffer

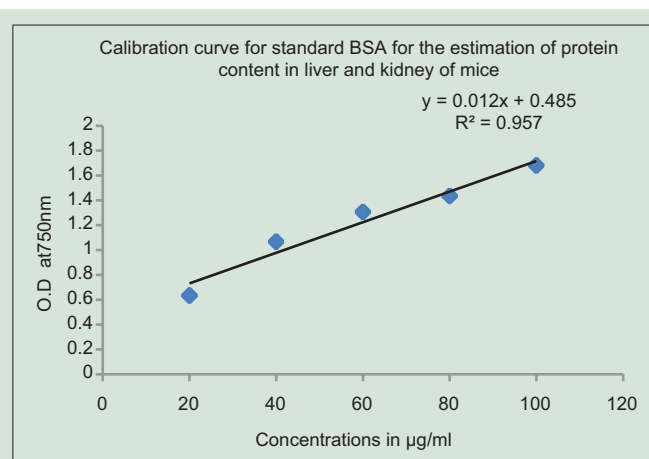


Figure 2: Standard calibration curve for bovine serum albumin to find out protein content

Table 1: Preliminary phytochemical screening of methanolic extract of *Curcuma caesia* Roxb.

| Chemical test | Result |
|--------------------------------|---------|
| Test for alkaloids | Present |
| Carbohydrate test | Present |
| Test for reducing sugar | Present |
| Test for flavonoids | Present |
| Test for terpenes and steroids | Present |
| Test for tannins | Present |

followed by the centrifugation at 10,000 rpm. To 0.1 mL of the enzyme source (supernatant), 1 mL of (0.12 M, pH = 7.2) phosphate buffer, 0.1 mL of 15 mM ethylenediaminetetraacetic acid, 0.1 mL of 10 mM sodium azide, 0.1 mL of GSSG, 0.6 mL of dH₂O were added, and the volume was made up to 1 mL with buffer. The reaction mixture was incubated for 3 min, followed by the addition of 0.3 mL of NADPH (9.6 Mm). The absorbance was read at 340 nm in spectrophotometer for every 15 s at an interval of 2–3 min. The enzyme activity will be expressed as μ moles of NADPH oxidized/minute/g tissue.

Protein estimation

Protein concentration was estimated following^[34]. 0.025 g of each tissue was homogenized in 1 mL of phosphate buffer saline. 0.5 mL of each

Table 2: ABTS + radical scavenging activity of a standard and methanolic extract of *Curcuma caesia* Roxb.

| Concentrations (µg/ml) | Gallic acid | | MECC | |
|-------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| | Mean±SD | Percentage of inhibition | Mean±SD | Percentage of inhibition |
| 5 | 0.98±0.002 | 53.79 | 1.472±0.004 | 30.59 |
| 10 | 0.872±0.003 | 58.88 | 1.698±0.003 | 30.69 |
| 20 | 0.792±0.002 | 62.65 | 1.253±0.004 | 40.92 |
| 40 | 0.774±0.002 | 63.5 | 1.115±0.039 | 47.43 |
| 60 | 0.624±0.002 | 70.57 | 0.985±0.005 | 53.6 |
| 80 | 0.153±0.006 | 92.78 | 0.984±0.002 | 63.5 |
| 100 | 0.139±0.003 | 93.44 | 0.747±0.004 | 64.78 |
| Regression equation | y=0.412x+51.84 | | y=0.381x+30.19 | |
| R | R ² =0.912 | | R ² =0.939 | |
| IC ₅₀ values | IC ₅₀ = -4.37 | | IC ₅₀ = 51.994 | |

SD: Standard deviation; IC₅₀: Inhibitory concentration; ABTS+: 2,2' azino bis (ethylbenzthiazolene-6-sulfonic acid) radical cation; MECC: Methanolic extract of rhizome of *Curcuma caesia* Roxb.

Table 3: Superoxide anion scavenging ability of standard and methanolic extract of *Curcuma caesia* Roxb.

| Concentrations (µg/ml) | Gallic acid | | MECC | |
|------------------------|----------------|--------------------------|----------------|--------------------------|
| | Mean±SD | Percentage of inhibition | Mean±SD | Percentage of inhibition |
| 20 | 0.274±0.004 | 86.19 | 0.559±0.004 | 71.83 |
| 40 | 0.266±0.008 | 86.59 | 0.539±0.011 | 72.84 |
| 60 | 0.234±0.007 | 88.21 | 0.530±0.015 | 73.29 |
| 80 | 0.227±0.004 | 86.04 | 0.522±0.009 | 73.7 |
| 100 | 0.185±0.004 | 90.68 | 0.508±0.005 | 74.4 |
| 120 | 0.177±0.005 | 91.08 | 0.475±0.011 | 76.07 |
| 140 | 0.174±0.002 | 91.23 | 0.474±0.017 | 76.12 |
| 160 | 0.159±0.002 | 91.98 | 0.445±0.025 | 77.58 |
| 180 | 0.128±0.003 | 93.55 | 0.400±0.118 | 79.84 |
| 200 | 0.118±0.005 | 94.05 | 0.317±0.004 | 84.03 |
| Regression equation | y=0.046x+84.81 | | y=0.057x+69.66 | |
| R | 0.889 | | 0.872 | |
| IC ₅₀ value | -756.73 | | -344.91 | |

SD: Standard deviation; IC₅₀: Inhibitory concentration; MECC: Methanolic extract of rhizome of *Curcuma caesia* Roxb.

Table 4: The effect of treatment with MECC on the micronuclei induced by CP in bone marrow cells of mice

| Treatment | Number of cells analyzed | MNPCEs | Percentage | Reduction percentage |
|----------------------------------|--------------------------|-----------|------------|----------------------|
| Water | 1000 | 4.6 | 0.46 | |
| Water + CP (50 mg/kg. b.wt) | 1000 | 153.6**** | 15.36 | |
| Solution 1 | 1000 | 3.6 | 0.36 | |
| Solution 2 | 1000 | 5.4 | 0.54 | |
| Solution 3 | 1000 | 5 | 0.5 | |
| Solution 1 + CP (50 mg/kg. b.wt) | 1000 | 89.4** | 8.94 | 41.77 |
| Solution 2 + CP (50 mg/kg. b.wt) | 1000 | 79.2* | 7.92 | 48.43 |
| Solution 3 + CP (50 mg/kg. b.wt) | 1000 | 48* | 4.8 | 68.75 |

The data in each group were pooled (n=5). *P<0.01, **P<0.005, ****P<0.002: Positive versus negative control (one-way ANOVA), *P<0.001 positive control versus other groups (one-way ANOVA). CP: Cyclophosphamide; MNPCEs: Micronucleated polychromatic erythrocytes; MECC: Methanolic extract of rhizome of *Curcuma caesia* Roxb. Solution 1: 100 mg/kg. b.wt of MECC, Solution 2: 250 mg/kg. b.wt of MECC, Solution 3: 500 mg/kg. b.wt of MECC

homogenate was diluted with 6 mL of phosphate-buffered saline and from this 0.5 mL of the diluted sample was used for the analysis. The reaction mixtures contain 0.5 mL of the homogenate, 0.7 mL of Lowry's solution. It was mixed through the vortex and incubated for 20 min. 0.1 mL Folin-Ciocalteu reagent was added and mixed in the vortex.

After 30 min of incubation absorbance was read at 750 nm against the reagent blank. The amount of protein was estimated from the standard calibration curve obtained using bovine serum albumin [Figure 2].

Statistical analysis

The results presented are expressed as mean ± standard deviation. The difference between treatment and control was analyzed by one-way ANOVA.

RESULTS

Preliminary phytochemical screening reveals the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, terpenes, steroids, and tannins in the methanolic extract of *C. caesia* Roxb. as indicated in Table 1.

The inhibitory effect of MECC against ABTS⁺ and superoxide dismutase (SOD) was compared with standard compound gallic acid [Tables 2 and 3]. The highest inhibitory effect of MECC against ABTS⁺ was found to be 64.78% at its highest concentration (100 µg/mL) which lies in between 63.5% and 72.7% at the concentrations of 40 µg/mL and 60 µg/mL of gallic acid. The IC₅₀ value of MECC is 59.99 µg/mL as compared to the IC₅₀ value of gallic acid with -4.37 µg/mL. On the other hand, the highest inhibition (84.03%) shown at 200 µg/mL of MECC against SOD was comparable to the value of gallic acid standard at 20 µg/mL (86.19%) [Tables 2 and 3].

An increase in the number of micronuclei was observed when treated with CP only as shown in Table 4. But, the pretreatment of different concentrations of MECC followed by CP reduces the micronuclei formation significantly (P < 0.005, P < 0.01, P < 0.001) (R² = 0.980). The

Table 5: Effect of MECC on biochemical parameters in CP induced hepatic toxicity

| Dose (mg/kg.b.wt) | SGOT (U/ml) | SGPT (U/ml) | LPO liver (nmole/g tissue) (%) | GSH liver (nmole of GSH/g tissue) | GR liver (µmoles of NADPH oxidized/min/g tissue) | Protein (liver) |
|---------------------------------|------------------------|---------------------------|--------------------------------|-----------------------------------|--|---------------------------|
| Water | 43±0.244 | 6±0.116 | 0.120 (78)±0.147 | 0.182±0.052 | 1.926±0.571 | 22.58±0.085 |
| Water + CP (50 mg/kg.b.wt) | 444±0.017* | 101.33±0.046 ^b | 0.550±0.255*** | 0.007±0.003 ^b | 0.0486±0.0152 | -13.75±0.092 ^b |
| Solution 1 + CP (50 mg/kg.b.wt) | 286±0.095* | 33.67±0.036 ^b | 0.253 (54)±0.076** | 0.0602±0.0478** | 1.274±1.084** | -3.33±0.065** |
| Solution 2 + CP (50 mg/kg.b.wt) | 185±0.175* | 23.33±0.082 ^a | 0.192 (65)±0.037* | 0.0606±0.0472** | 1.641±0.837*** | 6.25±0.085*** |
| Solution 3 + CP (50 mg/kg.b.wt) | 108±0.144 ^a | 18±0.152*** | 0.176 (68)±0.008** | 0.111±0.054*** | 1.695±0.861*** | 14.16±0.113 ^a |

The data in each group were pooled ($n=5$). * $P<0.01$, ** $P<0.05$, *** $P<0.005$, ^a $P<0.001$, ^b $P<0.0001$, ^c $P<0.02$. CP: Cyclophosphamide; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; LPO: Lipid peroxidation; GSH: Glutathione reduced; GR: Glutathione reductase; MECC: Methanolic extract of rhizome of *Curcuma caesia* Roxb.

Table 6: Effect of MECC on biochemical parameters in CP induced kidney toxicity

| Dose (mg/kg.b.wt) | LPO kidney (nmole/g tissue) (%) | GSH kidney (nmole of GSH/g tissue) | GR kidney (µmoles of NADPH oxidized/min/g tissue) | Protein kidney |
|---------------------------------|----------------------------------|------------------------------------|---|--------------------------|
| Water | 0.236 (74.78)±0.183 | 0.082±0.063 | 2.184±0.217 | 20.75±0.085 |
| Water + CP (50 mg/kg.b.wt) | 0.936±0.254* | 0.011±0.004** | 0.851±0.663*** | -7.33±0.105 ^a |
| Solution 1 + CP (50 mg/kg.b.wt) | 0.352 (62.39)±0.183*** | 0.025±0.006*** | 1.588±0.083** | 1.588±0.083** |
| Solution 2 + CP (50 mg/kg.b.wt) | 0.313 (66.55)±0.075* | 0.047±0.022* | 1.667±0.290** | 4.833±0.079** |
| Solution 3 + CP (50 mg/kg.b.wt) | 0.237 (74.67)±0.166 ^a | 0.065±0.033* | 1.921±0.076* | 12.25±0.110* |

* $P<0.01$, ** $P<0.05$, *** $P<0.005$, ^a $P<0.001$, ^c $P<0.02$, One-way ANOVA. CP: Cyclophosphamide; LPO: Lipid peroxidation; GSH: Glutathione reduced; GR: Glutathione reductase; MECC: Methanolic extract of rhizome of *Curcuma caesia* Roxb.

reduction percentage of micronuclei was 41.77%, 48.43%, and 68.75% at different concentrations (100, 250, 500 mg/kg. b.wt respectively). There was no sign of toxicity in the treatment with extracts only since the values were almost near to normal groups, and they are not significantly different from the NC groups [Table 4].

The PC group was compared with NC groups, and all other treatment groups were compared to the PC group.

Levels of SGOT and SGPT in positive control groups were found to be significant ($P < 0.01$, $P < 0.0001$) as compared to the normal groups [Table 5]. But their amount was found to be reduced with the administration of the extract at different concentrations. Peroxidation to the lipid membranes of both liver and kidney were increased in the CP treated mice, but the increased concentration was found to be reduced by 54%, 65%, 68% in the liver and 62.39%, 66.55%, 74.67% in kidney respectively at the tested concentrations of the extract, significantly as shown in Tables 5 and 6. *In vivo* antioxidant enzymes such as GSH, GR, and protein were also found to be decreased in the CP treated mice, however the pretreatment with extract increased their concentration near to normal, which is an indication of the protective effect of the extract against oxidative stress produced by the reactive metabolites of CP [Tables 5 and 6].

DISCUSSION

Medicinal plants and their derivatives have been used as an alternative to synthetic medicines in many countries. Medicinal plants play an important role in two-sided approach: One, plant-derived compounds are complex in nature which are difficult to synthesize in the laboratory and are helpful in the prevention of onset of cancer by its antioxidant activity and stimulation of the immune system,^[35] second, plant-derived compounds are used for prevention and decreasing side effects of conventional cancer treatments.^[36] The present study, on MECC reveals the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, terpenes, steroids, and tannins in it [Table 1]. The process of respiration, cell-mediated immune functions, and other process utilizing oxygen produces free radicals as an end product, continuously in the living body.^[37] Our body has enough antioxidant to defense against such free radicals, but exogenous as well as extra free radicals inside the

body imbalances defense system, leading to oxidative stress.^[38] Such oxidative stress is the leading cause of DNA damage and micronuclei formation in bone marrow cells.^[39] In the present study, MECC was found to scavenge ABTS⁺ and Superoxide anion free radicals [Tables 2 and 3] and such scavenging activity is regarded as one of the most important techniques in preventing damage to DNA.^[40] CP induced micronuclei in PCEs in the bone marrow cells of mice is an indication of chromosomal damage^[41-43] similar to the present study as shown in Table 4. There was a significant increase in micronuclei ($P < 0.002$) in the PC group as compared to normal groups. But the formation of micronuclei was reduced significantly ($P < 0.005$, $P < 0.01$, $P < 0.001$) with the pretreatment of MECC at different concentrations (100, 250, 500 mg/kg. b.wt) with the percent reduction of 41.77%, 48.43% and 68.75% respectively. CP under metabolic activation by cyt p450 produces metabolic products such as, hydroxycyclophosphamide which is used for chemoprevention^[44] and acrolein (ROS) that cross-links DNA, also decrease the antioxidant activity.^[45] A previous study on a methanolic extract of *C. caesia* Roxb. found to scavenge against 2,2-diphenyl-1-picrylhydrazyl.^[23,46] In addition to this, the present study also found MECC to scavenge against ABTS + and SO²⁻, based on these it was hypothesized that MECC being an anti-oxidative prevents the interaction of DNA and metabolic product acrolein produced by CP in the nucleus resulting in an increase reduction of micronuclei formation. Peroxidation of lipids produces an end product, malondialdehyde (MDA) which disrupts the cell membrane, thereby increasing permeability to ions.^[47,48] Hepatic damage results in the leakage of SGOT and SGPT into the serum resulting in their increased concentrations.^[49] SGOT and SGPT are the liver marker enzymes and elevated level of them is an indicative of loss of functional integrity of cell membranes in the liver.^[50] In the present study, the MDA level of the PC group was increased in the liver significantly ($P < 0.005$) as compared to normal groups [Table 5]. The level of SGOT and SGPT were found to increase in the serum of the PC group significantly ($P < 0.01$; $P < 0.0001$) as compared to normal groups [Table 5] may be due to increase of MDA level. It was found that the level of peroxidation in kidney was also found to increase significantly ($P < 0.02$) in PC groups as compared to normal groups. However, pretreatment with MECC at

three different concentrations (100, 250, 500 mg/kg, b.wt) reduces the formation of lipid peroxidation in both kidney and liver significantly as compared to positive groups as shown in the above Tables 5 and 6. Also, the level of SGOT and SGPT were also found to reduce significantly with different concentrations of MECC tested as shown in Table 5.

Determination of GSH is regarded as one of the most important factor to show the amount of antioxidant reserve in the organism.^[51-53] Reactive metabolites of CP (acrolein) conjugate with GSH resulting in the formation of glutathionylpropionaldehyde which induces oxidative stress and depletion of GSH.^[54] The depletion of GR and protein content is also related to the production of reactive metabolites of CP.^[55] In the present study, the content of GSH, GR and protein were found to decrease in the PC group significantly [Tables 5 and 6] in both liver and kidney as compared to normal groups. Moreover pretreatment with MECC increased the content of GSH, GR, and protein in both liver and kidney of mice.

CONCLUSIONS

Our present work demonstrated that methaolic extract of rhizome of *C. caesia* Roxb. has not shown any genotoxicity and reduces the genotoxicity caused by reactive metabolites of CP. It is shown for the first time that MECC has protective effect against genotoxicity induced by CP in bone marrow cells as well as protects against toxicity induced in the liver and kidney by CP. So it could be provided as one of the herbal supplementation in chemoprevention of CP to ameliorate the side effects of it.

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Conflicts of interest

There are no conflicts of interest.

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