Cyclophosphamide (CP) is a nitrogen mustard alkylating agent using as a first line of cancer-fighting agent in developing countries. Breakthrough in cancer prevention and treatment and they have been since they have profound active ingredients yielding important effects, the research on medicinal plants or herbs has been intensified, blood clots fatigue, and infection. Cure, but most treatments, experiences side effects such as miserable pain, cancer patients. Such treatments lengthened the life or may permanently carcinogenic virus. With an ever increasing cause of cancer due to diet, environment, and conventional drugs have been recommended for 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.

**INTRODUCTION**

With an ever increasing cause of cancer due to diet, environment, and carcinogenic virus 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. However, due to less toxic and adverse effects, the research on medicinal plants or herbs has been intensified 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. Cyclophosphamide (CP) is a nitrogen mustard alkylating agent used in various types of cancer chemotherapy, but the International Agency for...
Research Centre has identified it as a carcinogen for both animals and humans. CP therapy causes injuries to normal tissue and peroxidative damage to kidney and other vital organs in which side effects are supported by the reactive oxygen species (ROS) such as acrolein (ROS) and phosphoramide mustard (ROS) produced by the metabolic activation of CP in the liver by cytochrome P450 mix functional oxidase system. 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. Many of the worked on it like anti-fungal activity, smooth muscle relaxant and anti-asthmatic activity, bronchodilating activity, antioxidant activity, anti-inflammatory and central nervous system depressant activity, locomotor depressant, anti-convulsant, anthelmintic activity, anti-bacterial activity and anti-ulcer activity. Methanolic extract of the rhizome of Curcuma caesia Roxb. (MECC) has high phenol content, it is also a good source of antioxidant and antimutagenic activity. The rhizomes of the plant are also a rich source of many phytoconstituents such as essential oils with camphor, ar-turmerone, (Z) ocinene, ar-curcumene, 1,8-cineole, elemene, borneol, bornyl acetate, and curcumene etc. 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.

Drugs and chemicals
All the drugs and chemicals used in this experiment were procured from Sigma 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 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:

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

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

Superoxide dismutase assay
It was performed following 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:

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

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 administrated 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 administrated 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 administrated 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. 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...
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:\(^29\):

\[
\text{Reduction} (%) = \frac{\text{mean DI in A} - \text{mean DI in B}}{\text{mean DI in A} - \text{mean DI in C}} \times 100
\]

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 µL of 10 mM FeCl\(_3\) and incubated at 37°C for 30 min. After incubation 400 µL of TCA, 50 µL of BHT, 0.5 mL of TBA, and 50 µL 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 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 \(d\)\(_2\)O 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 µmoles 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

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±0.005 | 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

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 | 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 | 1000        | 89.4** | 8.94      | 41.77               |
| Solution 2 + CP | 1000        | 79.2* | 7.92      | 48.43               |
| Solution 3 + CP | 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<0.01 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.

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 (% w/w) | GSH liver (µmole of GSH/g tissue) | GR liver (µmole of NADPH oxidized/min/g tissue) | Protein liver (µg/g tissue) |
|-------------------|-------------|-------------|-------------------|-----------------------------------|-----------------------------------------------|---------------------------|
| 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* | 0.550±0.253*** | 0.007±0.003                      | 0.048±0.0152                                 | −13.75±0.092               |
| Solution 1 + CP (50 mg/kg.b.wt) | 286±0.095*   | 33.67±0.036* | 0.253 (54)±0.076** | 0.0602±0.0478**   | 1.274±0.084**                             | −3.33±0.065**             |
| Solution 2 + CP (50 mg/kg.b.wt) | 185±0.175    | 23.33±0.082* | 0.192 (65)±0.057* | 0.0606±0.0472**   | 1.641±0.837**                             | 6.25±0.085***             |
| Solution 3 + CP (50 mg/kg.b.wt) | 108±0.144a   | 18.0±0.152*** | 0.176 (68)±0.088* | 0.111±0.054*     | 1.695±0.861**                             | 14.16±0.113***            |

The data in each group were pooled (n=5). *P<0.01, **P<0.05, ***P<0.005, **P<0.001, P<0.0001, P<0.02. CP: Cyclophosphamide; SGOT: Serum glutamic pyruvic transaminase; SGPT: Serum glutamic oxaloacetic 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 (µmole/g tissue) | GSH kidney (µmole of GSH/g tissue) | GR kidney (µmole of NADPH oxidized/min/g tissue) | Protein kidney (µg/g tissue) |
|-------------------|-----------------------------|-----------------------------------|-----------------------------------------------|---------------------------|
| 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               |
| 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*       | 0.065±0.033*                     | 1.921±0.076*                                 | 12.25±0.110*              |

*P<0.01, **P<0.05, ***P<0.005, **P<0.001, 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; second, plant-derived compounds are used for prevention and decreasing side effects of conventional cancer treatments. 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. 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. Such oxidative stress is the leading cause of DNA damage and micronuclei formation in bone marrow cells. 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. CP induced micronuclei in PCEs in the bone marrow cells of mice is an indication of chromosomal damage and 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) 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 and acrolein (ROS) that cross-links DNA, also decrease the antioxidant activity. A previous study on a methanolic extract of C. caesia Roxb. found to scavenge against 2,2-diphenyl-1-picyrylhydrazyl. In addition to this, the present study also found MECC to scavenge against ABTS + and so reduced 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. Hepatic damage results in the leakage of SGOT and SGPT into the serum resulting in their increased concentrations. 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. 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. Reactive metabolites of CP (acrolein) conjugate with GSH resulting in the formation of glutathionypropionaldehyde which induces oxidative stress and depletion of GSH. The depletion of GR and protein content is also related to the production of reactive metabolites of CP. 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 *Curcuma 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.

Acknowledgments

The authors thank to the Plant Biotechnology Laboratory, Department of Biotechnology, Assam University, Silchar for providing all the equipments.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Cancer Research Fund/American Institute for Cancer Research. Food, Physical Activity, and the Prevention of Cancer: A Global Perspective. USA: AICR; 2007.
2. World Health Organization. World Cancer Report 2008. France: IARC; 2008.
3. McMullen M. 8 Common Surgery Complications. WebMD Feature. WebMD. 2013.
4. Johnson IT. Phytochemicals and cancer. Proc Nutr Soc 2007;66:207-15.
5. Sawadogo WR, Schumacher M, Teiten MH, Dicato M, Diederich M. Traditional West African pharmacopeia, plants and derived compounds for cancer therapy. Biochem Pharmacol 2012;84:1225‑40.
6. Johnson IT. Phytochemicals and cancer. Proc Nutr Soc 2007;66:207-15.
7. Takimoto CH, Calvo E, Pazdur R, Coia LR, Hoskins WJ. Principles of oncologic pharmacotherapy. In: Cancer Management, A Multidisciplinary Approach. 9th ed. 2005. p. 23-42.
8. IARC. IARC monograph on the evaluation of carcinogenicity: An update of IARC monographs. Vol. 1-42: International Agency for Research on Cancer; 1987.
9. Abraham P, Sugumar E. Enhanced PO1 activity in the kidneys of cyclophosphamide treated rats may play a protective role as an antioxidant against cyclophosphamide induced oxidative stress. ArchToxicol 2008;82:237-8.
10. Patel JM. Stimulation of cyclophosphamide-induced pulmonary microsomal lipid peroxidation by oxygen. Toxicology 1987;45:79-91.
11. Hales BF. Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramid mustard, and acrolein. Cancer Res 1982;42:3016-21.
12. Sladek NE. Metabolism of oxazaphosphorines. Pharmacol Ther 1988;37:301-55.
13. Leishangthem S, Dinendra SL. Study of some important medicinal plants found in Imphal-East district, Manipur, India. Int J Sci Res Publ 2014;4.
14. Banerjee A, Nigam SS. Antifungal activity of the essential oil of *Curcuma caesia* Roxb. Indian J Med Res 1976;64:1318-21.
15. Arulmozi DK, Sridhar N, Veeranjaneyulu A, Arora SK. Preliminary mechanistic studies on the smooth muscle relaxant effect of hydroalcoholic extract of *Curcuma caesia*. J Herb Pharmacother 2006;6:117-24.
16. Paliwal P, Pancholi SS, Patel RK. Pharmacognostic parameters for evaluation of the rhizomes of *Curcuma caesia*. J Adv Pharm Technol Res 2011;2:56-61.
17. Mangia M, Shuaib M, Jain J, Kashyap M. In-vitro evaluation of antioxidant activity of *Curcuma caesia* Roxb. Int J Pharm Sci Res 2010;1:98-102.
18. Karmakar I, Saha P, Sarkar N, Bhattacharya S, Haldar PK. Neuropharmacological assessment of *Curcuma caesia* rhizome in experimental animal models. Orient Pharm Exp Med 2011;11:251-5.
19. Gill R, Kalsi V, Singh A. Phytochemical investigation and evaluation of antihemophilic activity of *Curcuma amada* and *Curcuma caesia* – A comparative study. Invent Impact Ethnopharmacol 2011:2011.
20. Rajamma AG, Bai V, Nambisian B. Antioxidant and antibacterial activities of oleoresins isolated from *Curcuma* species. Phytopharmacology 2012;5:312-7.
21. Das S, Bordoloi PK, Phukan D, Singh SR. Study of the anti-ulcerogenic activity of the ethanolic extracts of *Curcuma caesia* eecq against gastric ulcers in experimental animals. Asian J Pharm Clin Res 2012;5:200-3.
22. Krishnaraj M, Manibhushanrao K, Mathivanan N. A comparative study of phenol content and antioxidant activity between non conventional *Curcuma caesia* Roxb. and *Curcuma amada* Roxb.Int J Plant Prod 2010;4:169-74.
23. Devi HP, Mazumder PB, Devi LP. Antioxidant and Antimutagenic activity of *Curcuma caesia* Roxb. rhizome extracts. Toxicol Rep 2015;2:423-8.
24. Pandey AK, Choudhary AR. Volatile constituents of rhizome oil of *Curcuma caesia* Roxb. from central India. Flavour Fragr 2003;18:463-5.
25. Sofowora A. Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd.; 1993. p. 134-56.
26. Re R, Pellegrini N, Protegente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
27. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972;46:849-54.
28. Schmid W. The micronucleus test. Mutat Res 1975;31:9-15.
29. Serpeloni JM, Bissaro dos Reis M, Rodrigues J, Campaner dos Santos L, Vilegas W, Varanda EA, et al. In vivo assessment of DNA damage and protective effects of extracts from *Miconia* species using the comet assay and micronucleus test. Mutagenesis 2008;23:501-7.
30. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
31. Halliwell B, Gutteridge JM. The chemistry of free radicals and related reactive species. In: Free Radicals in Biology and Medicine. 2nd ed. Oxford, UK: Oxford University Press; 1989. p. 60-1.
32. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582:67-78.
33. David M, Richard JS. In: Bergmeyer J, Grab M, editors. Methods of Enzymatic Analysis. Deerfield Beach, Florida: Verlag Chemie, Wenhien, 1983. p. 358.
34. Lowry OH, Rosebrough NJ, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
35. Mantle D, Wilkins RM. Medicinal plants in the prevention and therapy of cancer. In: Yaniv Z, Bachrach U, editors. Handbook of Medicinal Plants. New York: The Spectrum Books Ltd.; 1993. p. 134-56.
Heisanam Devi and Pranab Mazumder: Extract of *Curcuma caesia* Roxb. Prevents by Cyclophosphamide to Bone Marrow Cells, Liver and Kidney

Pharmacognosy Research, Vol 8, Issue 1, Jan-Mar, 2016

37. Hussain A, Ramteke A. Flower extract of *Nyctanthes arbor-tristis* modulates glutathione level in hydrogen peroxide treated lymphocytes. Pharmacognosy Res 2012;4:230-3.

38. Gangar SC, Sandhir R, Koul A. Anti-clastogenic activity of *Azadirachta indica* against benzo (a) pyrene in murine forestomach tumorigenesis bioassay. Acta Pol Pharm 2010;67:381-90.

39. Jain R, Jain SK. Effect of *Buchanania lanzan* Spreng. bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice. Asian Pac J Trop Med 2012;5:187-91.

40. Hosseinimehr SJ, Karami M. Chemoprotective effects of captopril against cyclophosphamide-induced genotoxicity in mouse bone marrow cells. Arch Toxicol 2005;79:482-6.

41. Vijayalaxmi RRJ, Reiter RJ, Herman TS, Meltz ML. Melatonin and radioprotection from genetic damage: *in vivo* /*in vitro* studies with human volunteers. Mutat Res 1996;371:221-8.

42. Tice RR, Ereksen GL, Shelby MD. The induction of micronucleated polychromatic erythrocytes in mice using single and multiple treatments. Mutat Res 1990;234:187-93.

43. MacGregor JT, Schlegel R, Choy VN, Wehr CM. Micronuclei in circulating erythrocytes: A rapid screen for chromosomal damage during routine toxicity testing in mice. Dev Toxicol Environ Sci 1983;11:555-8.

44. Huttunen KM, Raunio H, Rautio J. Prodrugs – from serendipity to rational design. Pharmocol Rev 2011;63:750-71.

45. Ray S, Pandit B, Ray SD, Das S, Chakraborty S. Cyclophosphamide induced lipid peroxidation and changes in cholesterol content: Protective role of reduced glutathione. Iran J Pharm Sci 2011;7:255-67.

46. Chiragini P, Sharma GJ, Sinha SK. Sulfur free radical reactivity with curcumin as reference for evaluating antioxidant properties of medicinal Zingiberaceae. J Environ Pathol Toxicol Oncol 2004;23:227-36.

47. Devasagayam TP, Boloor KK, Ramasarma T. Methods for estimating lipid peroxidation: An analysis of merits and demerits. Indian J Biochem Biophys 2003;40:300-8.

48. Anoopkumar-Dukie S, Walker RB, Daya S. A sensitive and reliable method for the detection of lipid peroxidation in biological tissues. J Pharm Pharmacol 2001;53:263-6.

49. Deb AC. Fundamentals of Biochemistry. 7th ed. Kolkata: New Central Book Agency; 1998.

50. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. Drug Chem Toxicol 1978;1:163-71.

51. Lu SC, Huang ZZ, Yang JM, Tsukamoto H. Effect of ethanol and high-fat feeding on hepatic gamma-glutamylcysteine synthetase subunit expression in the rat. Hepatology 1999;30:209-14.

52. Odukoya OA, Inye-Agha SI, Illori OO. Immune boosting herbs: Lipid peroxidation in liver homogenates as index of activity. J Pharm Pharmacol 2007;2:190-5.

53. Balouchzadeh A, Rahimi HR, Ebadollahi N, Minei-Zangi AR, Sabzevari O. Aqueous extract of Iranian green tea prevents lipid peroxidation and chronic ethanol liver toxicity in rats. J Pharm Pharmacol 2011;6:691-700.

54. Pastore A, Piemonte F, Locatelli M, Lo Russo A, Gaeta LM, Tozzi G, et al. Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. Clin Chem 2001;47:1467-9.

55. Rehman MU, Tahir M, Ali F, Qamar W, Latteef A, Khan R, et al. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: The protective effect of ellagic acid. Mol Cell Biochem 2012;365:119-27.

ABOUT AUTHORS

Heisanam Pushparani Devi, She is a Research Scholar in the Department of Biotechnology, Assam University Silchar and her interest is on medicinal plant studies like antioxidant activity, antimutagenic activity, antigenotoxic activity etc.

Pranab Behari Mazumder, Professor Pranab Behari Mazumder is a faculty of Department of Biotechnology, assam University, Silchar and his interest is on medicinal plant studies as well microbial related studies. Currently many of the Researchers is working under his guidance and many of them have awarded Ph. D degree under his Guidance.