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Antioxidant and antimutagenic activity of *Curcuma caesia* Roxb. rhizome extracts

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ABSTRACT

The rhizomes of *Curcuma caesia* Roxb. (zingiberacea) are traditionally used in treatment of various ailments and metabolic disorders like leukoderma, asthma, tumours, piles, bronchitis, etc. in Indian system of medicine. Considering the importance of natural products in modern phytomedicine, the antioxidant and antimutagenic activities of *C. caesia* Roxb. rhizome extract and its fractions were evaluated. The ethanolic fraction showed highest antioxidant activity by DPPH assay (86.91%) comparable to ascorbic acid (94.77%) with IC50 value of 418 μg/ml for EECC followed by MECC (441.90 μg/ml) > EAECC(561 μg/ml) > AECC(591 μg/ml). Based on the antioxidant activity, three of the rhizome extracts were evaluated for their antimutagenic properties against indirect acting mutagen cyclophosphamide (CP) using *Salmonella typhimurium* strains TA98 and TA100. The antimutagenic activity of the extracts against indirect acting mutagen cyclophosphamide in the presence of mammalian metabolic activation system was found to be significant ($p < 0.01$, $p < 0.05$). All the extracts showed similar antimutagenicity in dose dependent manner. The total phenolic content as well as reducing ability of the extracts was also determined.

1. Introduction

The emerging concepts of cancer is that the cancer cells are unstable and its unstability is brought about by the documentations of cascade of mutations caused by mutagens and suggested that mutagenesis drives out tumour progression [1]. Mutations results from the side effects of free radicals such as hydrogen peroxides, superoxide anions, and organo peroxides, etc. produced by drugs, ultraviolet radiations, ionising radiations, pollution as well as the endproducts of normal metabolic process of aerobic organisms [2–4]. The interaction of the free radicals with polyunsaturated fatty acids, nucleotides and disulphide bonds [5] has been implicated as the major factor to cause the oxidation of the biological compounds and

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eventually leads to mutations [6] and many degenarative diseases like emphysema, cardiovascular, inflammatory diseases, cataracts, etc. [5]. Cellular system has developed many endogenous antioxidants such as superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases and reductase, and nonenzymatic antioxidants like vitamin E (tocopherols and tocotrienols), vitamin C, etc. [7] to neutralise the free radicals [8]. This has triggered to search for effective antioxidant agents from various sources including plants. Many researchers have investigated that the increase levels of antioxidants present in plants are believed to decrease the oxidative damage and its harmful effects [9]. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are preferable but can cause serious ill effects in human health as per recent reports Lobo et al. [10]. The use of plants and medicinal plants has been recommended to combat the effect of free radicals/mutagens because they can induce phase II enzymes reducing the action of initiation, promotion or progression stages of cancer and other degenerative diseases [11–14]. Also the plants are rich source of secondary metabolites such as flavonoids, phenolics, carotenoids, coumarins, anthraquinones, tannins, terpenoids, saponins that play a prominent role in inhibiting human carcinogenesis and repair the cell mutations [15].

Curcuma caesia Roxb. (black turmeric) is a perennial herb with bluish black rhizomes and it is famous for its medicinal properties. It is recognised as a medicinal herb to possess with various properties such as anti-fungal activity Banerjee and Nigam [16], smooth muscle relaxant and anti-asthmatic activity Arulmozhi et al. [17], bronchodilating activity Paliwal et al. [18], antioxidant activity Mangla et al. [19], anxiolytic and CNS depressant activity, locomotor depressant, anti-convulsant Karmakar et al. [20], anthelmintic activity Gill et al. [21], anti-bacterial activity Rajamma et al. [22], anti-ulcer activity Das et al. [23]. The phytochemical studies of *C. caesia* revealed the presence of multiple phytoconstituents like essential oils with camphor, ar-turmerone, (Z) ocemene, ar-curcumene,1,8-cineole, elemene, borneol, bornyl acetate, curcumene, etc. [24]. To the best of our knowledge there is no report available on the antimutagenic activity of *C. caesia* Roxb. Therefore we have selected the rhizome of this plant and evaluated the antioxidant and antimutagenic activity of some of the selected extracts against indirectly acting mutagen cyclophosphamide.

2. Materials and methods

2.1. Plant material collection and extraction

Rhizomes of *C. caesia* Roxb. were collected in the month of November 2012, from the region of Nambol, Bishnupur District, Manipur, India. The rhizomes were cut into pieces and sun dried. The dried rhizomes were coarsely powdered and 100 g of it was successfully extracted with various solvents starting from least polar solvents to more polar, i.e. from petroleum ether to ethyl acetate, ethanol, methanol and then finally to water through soxhlet at a temperature of 50–60 °C for a period of 12–24 h. The crude extracts of each solvent were dried in water bath and kept for further uses.

2.2. DPPH radical scavenging activity

The quenching of free radical activity of different extracts were determined by spectrophotometric method against 2,2-diphenyl-1-picryl hydrazyl (DPPH) following [25]. 1 ml of each extract of various concentrations (25–800 μg/ml) were mixed with 1 ml of DPPH (0.1 mM) solution prepared in ethanol and incubated in dark for 20 min and absorbance values were recorded at 517 nm. 1 ml of ethanol and 1 ml of ethanolic solution of DPPH (0.2 mM) was taken as control. Similarly 1 ml of ethanolic solution of ascorbic acid (200 μg/ml) was mixed with 1 ml of DPPH ethanolic solution and absorbance values were recorded. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity}\,(\%) = \frac{A_b - A_a}{A_b} \times 100$$

where A_b is the absorption of the blank and A_a is the absorption of the extract sample.

2.3. Determination of total phenolic contents in the plant extracts

The concentration of phenolics in plant extracts was determined using Folin Ciocalteau method [26] with little modifications. The extracts in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of each extract solution, 2.5 ml of 10% Folin–Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% $NaHCO_3$. Blank was prepared, containing 0.5 ml ethanol, 2.5 ml 10% Folin–Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated at 45 °C for 45 min. The absorbance were determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was calculated from the calibration line; then the content of phenolics in each extracts was expressed in terms of gallic acid equivalent (mg of GAE/100 g d.w. of extract).

2.4. Reducing power assay

The ability to reduce ferric ions to ferrous ions by the antioxidants present in rhizomes of *C. caesia* Roxb. was determined by the method of Oyaizu [27] with little modification. From the different concentrations of each extract solutions (200–1000 μg/ml), 1 ml of each was mixed with 2.5 ml of 0.2 M of phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was heated at 50 °C for 20 min and then cooled followed by the addition of 2.5 ml of 10% TCA and then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was again mixed with 2.5 ml of distilled water and 0.5 ml of 0.5% $FeCl_3$ and the

absorbance was recorded at 700 nm against blank without extract. Increase in the absorbance values shows the increasing reducing ability of the extracts. The entire test was performed in triplicate.

2.5. Bacterial strains

Salmonella typhimurium strains TA98 and TA100 which are histidine-requiring mutants, were kindly provided by IMTECH, Chandigarh, India and are maintained as described by Maron and Ames [28]. The genotypes of the test strains were checked routinely for their histidine requirement, rfa mutatios, UV sensitivity (uvrB mutation). They were stored at −80 °C for further use.

2.6. S9 preparation

S9 is the mitochondrial enzyme mix required for metabolic activation of indirect acting mutagens like cyclophosphamide. The S9 mixture was prepared from male rat liver using the chemicals 1 M glucose-6-phosphate, 0.1 M NADP, 0.2 M phosphate buffer, 0.4 M $MgCl_2$ + 1.65 M KCl (Himedia – India) as described by Maron and Ames [28]. S9 mix was prepared fresh for each assay.

2.7. Salmonella-microsome assay

The bacterial strains were incubated in Nutrient Broth for 16 h at 37 °C in an orbital shaker to obtain a density of 2×10^9 colony forming units (CFU/ml). 0.1 ml of an overnight culture of bacteria and 0.5 ml of sodium phosphate buffer (0.2 M, pH 7.4 for assay without S9) supplemented with 0.2 mM L-histidine and 0.2 mM D-biotin solution containing different concentrations of each extract. They were mixed using votexer for 10 min. The resulting complete mixture was poured on minimal agar plates prepared as described by Maron and Ames [28]. The plates were incubated at 37 °C for 48 h and the revertant bacterial colonies of each plate were counted. Data were collected with a mean ± standard deviation of three experiments ($n = 3$).

2.8. Antimutagenicity testing

For the experiment with S9 mix, 0.1 ml of overnight grown bacterial cultures were taken followed by the addition of 0.2 mM. Histidine-Biotin solution supplemented with each extracts at different concentrations, were mixed and incubated for 3 min. After incubation 0.1 ml of the CP (500 μg/plate) and 0.5 ml of S9 mix were added. The experiment was performed as mentioned above. Percentage inhibition was calculated using the formula [29].

$$\%\,\text{Inhibition of mutagenicity} = \frac{(R1 - SR) - (R2 - SR)}{R1 - SR} \times 100$$

where $R1$ is the number of revertants without extracts but with CP, $R2$ the number of revertants with extracts plus mutagen and SR is the spontaneous revertants i.e. without extracts and mutagen.

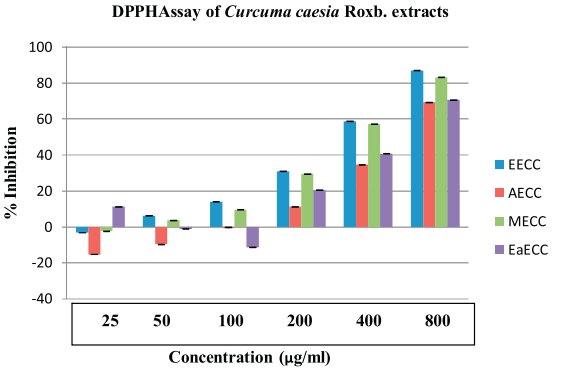

Fig. 1. DPPH method. EECC: ethanolic extract of *Curcuma caesia* Roxb., MECC: methanolic extract of *Curcuma caesia* Roxb., EaECC: ethyl acetate extract of *Curcuma caesia* Roxb., AECC: aqueous extract of *Curcuma caesia* Roxb.

2.9. Statistical analysis

The results are presented as the average and S.D. (standard deviations) of three experiments with triplicate plates/dose/experiment. The regression analysis was carried out in Microsoft Excel 2007 between % inhibition of mutagenicity and values of concentrations of the plant extracts. Student's t test was performed to compare the mean values with the positive control.

3. Results

3.1. Antioxidant activity

The free radical scavenging activity of the rhizome extracts of *C. caesia* Roxb. was measured as decolorizing activity following the trapping of the unpaired electron of DPPH as shown in Fig. 1. The fractions showed a varied free radical scavenging activity. The ethanol fraction was found to be the most active free radical scavenger exhibited (86.914% decrease at a concentration of 800 μg/ml) compared to ascorbic acid (94.770%). Likewise the crude methanolic, ethyl acetate and aqueous extract showed scavenging activity with a percent decrease of 83.104%, 70.44% and 69.19%. The IC50 value ranges in the order of 418 μg/ml (EECC) > 441.90 μg/ml (MECC) > 561 μg/ml (EAECC) > 591 μg/ml (AECC), the lowest being the highest antioxidant activity. The ethanolic extract neutralised 50% of free radicals at the concentration of 418 μg/ml (Fig. 2).

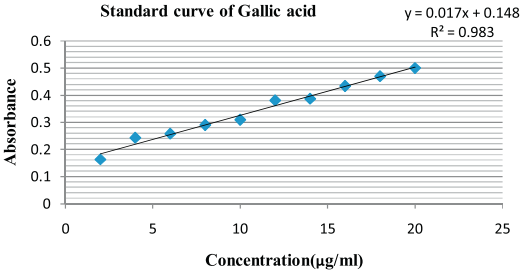

Fig. 2. Standard curve of gallic acid to find out the total phenolic content.

Table 1
Reducing power of the extracts.

Conc (μg/ml)	EECC	MECC	EaECC	AECC
1000	2.480 ± 0.010	1.639 ± 0.029	0.899 ± 0.053	0.348 ± 0.023
800	2.277 ± 0.068	1.368 ± 0.029	0.468 ± 0.028	0.275 ± 0.015
500	1.511 ± 0.041	0.788 ± 0.005	0.333 ± 0.022	0.180 ± 0.009
200	0.775 ± 0.002	0.372 ± 0.001	0.159 ± 0.013	0.074 ± 0.002
Ascorbic acid (200 μg/ml)	2.425 ± 0.03			

Table 2
Number of his+ revertants in *Salmonella typhimurium* strains produced by *Curcuma caesia* Roxb. extracts against cyclophosphamide.

Treatment conc. (μg/ml)	TA98		TA100	
	−S9	+S9	−S9	+S9
S.R	92.66 ± 6.94	304 ± 23	55 ± 4.54	213 ± 13.06
P.C: 50	299.66 ± 26.44	718 ± 94	135.66 ± 19.36	652.66 ± 71.2
EECC: 50	116.33 ± 18.14	395.11 ± 71.2*	98 ± 10.42	398.66 ± 64.8*
500	111.33 ± 19.14	386.51 ± 74*	80.33 ± 10.63	379.33 ± 63.8*
5000	104.33 ± 21.14	341.45 ± 93.72*	71 ± 16.63	334.66 ± 86.51*
MECC: 50	179.66 ± 39.98	491.67 ± 98.28*	126.66 ± 3.39	434.33 ± 44.93*
500	163.66 ± 47.6	487.66 ± 56*	105.66 ± 5.39	412.33 ± 43.4*
5000	144.66 ± 13.55	401.66 ± 93.14*	91.66 ± 10.17	385.33 ± 74*
ECC: 50	221.66 ± 15.9	596.67	131.33 ± 3.86	466.33 ± 21.7*
500	211.33 ± 22.89	562.67 ± 3.5**	121 ± 5.54	449.66 ± 45.7*
5000	95.66 ± 31.56	479.66 ± 33.15*	97.66 ± 9.74	410.66 ± 51.8*

The data represented in the table is the mean ± S.D. values of three replicates.
* $p < 0.01$.
** $p < 0.05$.
EECC: ethanolic extract of *Curcuma caesia* Roxb.; MECC: methanolic extract of *Curcuma caesia* Roxb.; AECC: aqueous extract of *Curcuma caesia* Roxb.; P.C: positive control; C.P: cyclophosphamide; S.R: spontaneous revertants.

3.2. Total phenolic content

The total phenolic contents in the examined plant extracts using the Folin Ciocleu's reagent is expressed in terms of gallic acid equivalent (the standard curve: $y = 0.0178x + 0.148$; $R^2 = 0.9831$). Total phenolic contents in the examined extracts ranged from MECC = 52.11 mg/100 g d.w., EECC = 68.64 mg/100 g d.w., EaECC = 38 mg/100 g d.w., AECC = 4.82 mg/100 g d.w. of the extract. The highest concentration of phenols was measured in ethanolic followed by methanolic, ethyl acetate and aqueous extracts.

The reducing power of *C. caesia* Roxb. rhizome extracts was dose dependent and is presented in the following table. The maximum absorbance of ethanolic extracts at 1000 μg/ml is more or near to ascorbic acid at 200 μg/ml as given in Table 1. Reducing power methods indirectly evaluates the antioxidant activity (Qureshi et al. [51]) (Table 2).

The increase in the absorbance indicates an increase in reductive ability [30].

Based on the promising antioxidant and reducing activity, ethanolic, methanolic and aqueous extracts were evaluated for their antimutagenic activity by Ames test against indirect acting mutagen cyclophosphamide. All the extracts were found to inhibit in dose dependent manner. Linear relationship between extract dose and antimutagenic response in the case of EECC without S9 is strong in the strain TA98 ($r^2 = 0.99$) followed by TA100 ($r^2 = 0.97$), with S9 it is strong in the strain TA98 ($r^2 = 0.99$) followed by TA100 ($r^2 = 0.95$). At all the doses antimutagenic response was significant at ($p < 0.01$) against both the strains with a percent mutagenicity decrease from 77.99 to 90.95 for TA98 followed by TA100 with percent antimutagenicity starting from 57.77 to 72.32. Similar trend was followed for methanoilc extract of *C. caesia* Roxb. Linear relationship between extract dose and antimutagenic response in the case of MECC without S9 is strong in the strain TA98 ($r^2 = 0.99$) followed by TA100 ($r^2 = 0.97$), with S9 it is strong in the strain TA98 ($r^2 = 0.99$) followed by TA100 ($r^2 = 0.86$). At all the doses antimutagenic response was significant at ($p < 0.01$) with the percent mutagenicity decrease from 54.66 to 76.41 in case of TA98 followed by TA100 with the percent mutagenicity decrease from 49.65 to 60.80 in MECC. The significant level shown was ($p < 0.01$) for all concentrations 50 μg, 500 μg and 5000 μg. Linear relationship between extract dose and antimutagenic response in the case of AECC without S9 is strong in the strain TA98 ($r^2 = 0.98$) followed by TA100 ($r^2 = 0.95$), with S9 it is same for both the strain TA98 ($r^2 = 0.95$) and TA100 ($r^2 = 0.95$). At the dose of 50 μg of AECC antimutagenic response was insignificant with percent inhibition of 29.30 but at 500 μg the antimutagenic response was significant at ($p < 0.05$) with percent inhibition of 37.51 and at the dose 5000 μg it was significant at ($p < 0.01$) (57.57% inhibition) in case of TA98 and in case of TA100 in AECC the significant level shown was ($p < 0.01$) for all concentrations with the percent mutagenicity decrease from 29.30 to 57.57.

4. Discussion

Considerable attention has been focussed in recent years on the exploration of phytotherapeutic agents for

the treatment of oxidative stress and mutation related disorders. The use of medicinal plants is perhaps the oldest method of coping with illness. They can be easily metabolised inside the body without any harmful effects that leads to the phytochemical based remedies [31–34]. Reactive oxygen intermediates like superoxides, hydrogen peroxides and hydroxyl radicals are known to mediate macromolecular damages by reacting with nucleic acids, proteins as well as various membrane components thus act as direct and indirect initiators of mutagenesis and carcinogenesis [35]. On the basis of this it has been hypothesised that the involvement of antioxidant might be considered as the safest approach in the prevention of process leading to mutagenesis. The chemistry of free radicals is complicated and it caused a major limitation in the identification of free radical scavenging activity. To withstand this problem the potential antioxidant substance are tested in in vitro model and such approaches expand the scope of antioxidant activity. The mechanism that contributes to the antioxidant capacity of phenols and flavanoids include free radical scavenging ability, hydrogen or electron donation ability, chelation of redox active metals ions, modulation of gene expression and interaction with the cell signalling pathways [36]. Therefore, we have examined the rhizome extracts of *C. caesia* Roxb. for antioxidant activity by DPPH free radical scavenging assay, total phenolic content and reducing power assay. The use of DPPH assay provides an easy and a rapid way to evaluate antioxidants by spectrophotometer [37]. The purple colour of DPPH reduces to light yellow with the intervention of plant extract; the most probable mechanism of action was hydrogen donation by the extracts [38]. Out of the four different extracts of rhizome DPPH radical scavenging activities follows in the order of EECC > MECC > EaECC > AECC with their percentage of inhibition ranging from −15.27 to 86.91%. In fact, the tested extracts are the complex mixtures of several compounds, particularly phenolic compounds which have diverse chemical structures that determine various properties. Rich source of phenolics are of increasing interest nowadays because they retard the oxidative degradation of bio molecules [39]. The chemical structure of phenolic compound which has hydroxyl group attached to benzene ring in its structure provides them the ability to act as free radical scavenger [40]. When reactive oxygen species are present at a certain concentration the bond between O and H is broken. The released hydrogen ion is made available to nucleophilic radicals which subsequently quenched their free radicals [42]. The phenol content of the *C. caesia* Roxb. extracts was found to be 52.11 mg/GAE for MECC, 68.64 mg/GAE for EECC, 38 mg/GAE for EaECC and 4.82 mg/gGAE for AECC in 100 g of dry weight of the extract in the present study. Literature reviews of Sarangthem and Haokip [41] also confirms that maximum curcuminoids, oil content, flavonoids, phenolics, different important amino acids, protein and high alkaloids are contained in the rhizome of this species. Antioxidants have been reported to act as scavengers of singlet oxygen and free radicals in biological systems [42,43]. As stated by Oyaizu [27], plant extracts has the reducing ability to transform Fe^{3+} to Fe^{+2} and reductones are responsible for it [44]. They have been found to exert antioxidant activity by breaking the free radical chains by donating a hydrogen atom [45]. The reducing power of extracts of *C. caesia* Roxb. was found to be remarkable and each extract was found to rise as the concentration gradually increases. The reducing power of the extracts follows the order EECC > MECC > EaECC > AECC as shown in the table as well as in the graph.

The antioxidant properties of phytochemicals are linked to their ability to scavenge free radicals generated either endogenously or by exogenous agents. These preventive agents can inhibit the mutation and cancer initiation process by modulating phase I and phase II enzymes, by blocking reactive species either by scavenging, electron donation or through chelation and thus maintains the DNA structure. The inhibition of mutagenesis are grouped into two namely desmutagens and bioantimutagens. It has been hypothesised that bioantimutagens act as second stage inhibitors that blocks the mutagen before they could attack the DNA [46] and bioantimutagenic effect of phytochemicals is determined in co incubation method [47]. The different extracts of *C. caesia* Roxb. have shown the following order of antimutagenicity EECC > MECC > AECC; against indirect acting mutagen cyclophosphamide (500 μg/plate). The results were based on the number of induced revertant colonies detected. According to Ames et al. [48], a compound is classified as a mutagen if it is able to increase at least twice the number of revertants as compared to spontaneous revertants. Earlier Morffi et al. [49] have reported the antimutagenic activity of *Magnifera indica* against CP in the strain TA100. Higher mutagenicity was found when CP was activated with S9 but inhibition of this microsomal activity was observed in the presence of rhizome extract. The present results showed the antimutagenic activity in Ames test that may be attributed in part to powerful radical scavenger associated with the extract. According to Negi et al. [50], a compound is found to possess its less antimutagenic activity if its percentage of inhibition is less than 25%, a moderate activity if the percentage inhibition value lies between 25% and 40% and a strong antimutagenicity effect if it is more than 40%. Ethanolic extract reduces the mutagenicity caused by indirect acting mutagen cyclophosphamide by 97.21% and 90.30% respectively in the strains TA98 and TA100 (in the presence of S9) at the highest tested dose (5000 μg/plate) which shows strong antimutagenic activity. From the results it was found that all the extracts showed strong effective antimutagenicity against cyclophosphamide.

5. Conclusion

In conclusion, the present study has shown for the first time that *C. caesia* Roxb. rhizome extract is a promising source for its antimutagenic compounds. Further studies are needed to isolate the active principles present in it. The present work supported the increasing evidence that the rhizome extract of *C. caesia* Roxb. plays an important role in cancer chemoprevention, particularly in defending cells from DNA damage induced by oxidative mutagens and by inhibiting CYP enzymes as documented in the present study.

Conflict of interest

The authors have no conflict of interest to this reputed journal.

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ORIGINAL ARTICLE

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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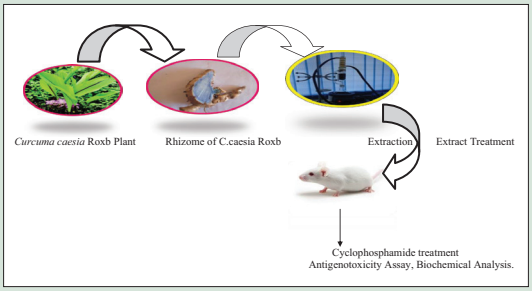
ABSTRACT

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 (ethylbenzthiazolene-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 cyclophosphophosphamide (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 phosphoramide mustard (ROS)[10] produced by the metabolic activation of CP in the liver by cytochrome $P4_{50}$ 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

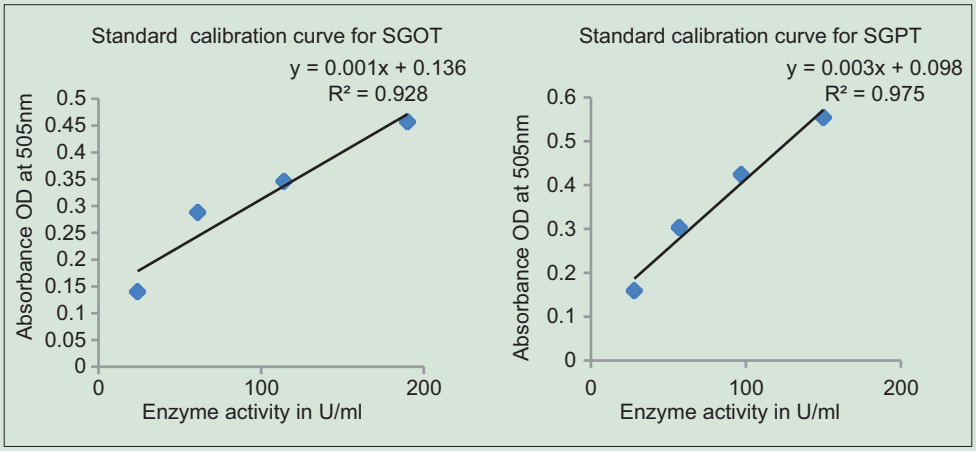

Figure 1: Standard calibration curve for serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase

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]:

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

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 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

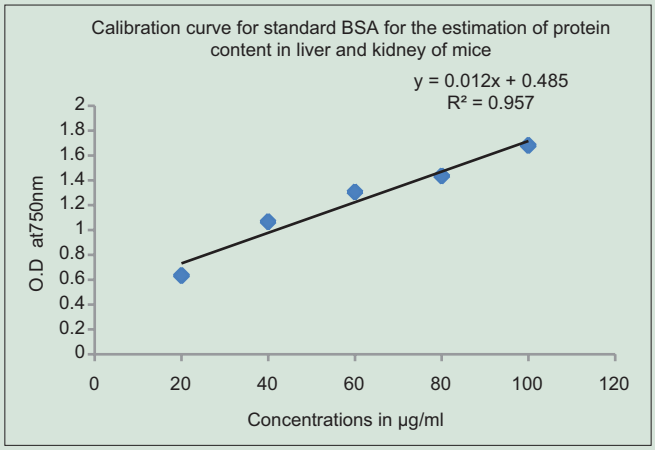

Figure 2: Standard calibration curve for bovine serum albumin to find out protein content

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 dH_2O 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		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; IC_{50}: 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; IC_{50}: 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-Ciocalteau 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 IC_{50} value of MECC is 59.99 µg/mL as compared to the IC_{50} 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 ($P < 0.005$, $P < 0.01$, $P < 0.001$) ($R^2 = 0.980$). 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, [#]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[a]	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, [#]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 SO^{2-}, 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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