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ORIGINAL ARTICLE

Safety Profile Investigations of *Meyna spinosa* (Roxb.) and *Oroxylum indicum* (Linn.) Extracts Collected from Northeast India

Shweta Singh[1,2], Pronobesh Chattopadhyay[1], Sahindra Kumar Borthakur[2], Rudragoud Policegoudra[1]

[1]Division of Pharmaceutical Technology, Defence Research Laboratory, Tezpur, [2]Department of Botany, Gauhati University, Guwahati, Assam, India

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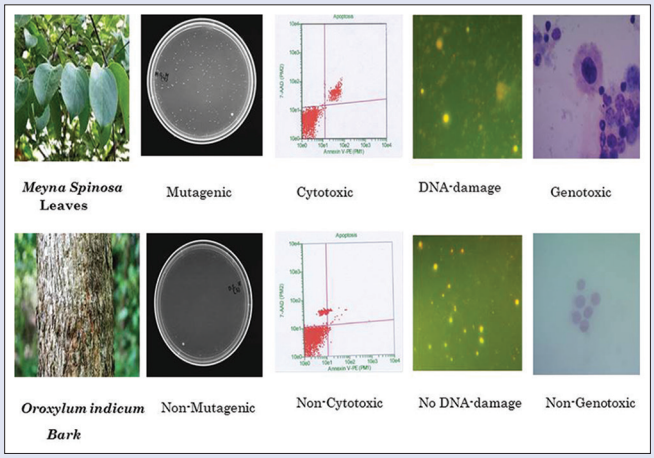
ABSTRACT

Background: *Meyna spinosa* (M.S) (Roxb.) ex Link and *Oroxylum indicum* (O.I) (Linn.) Vent, widely used traditional Northeast Indian medicinal plant used for various purposes, have not yet explored for safety profile. **Objective:** To investigate the safety profile of M.S (Roxb.) ex Link leaves and O.I (Linn.) Vent stem bark extracts collected from Northeast region of India. **Materials and Methods:** In this study, mutagenic, cytotoxic, and genotoxic and/or nontoxic potential of these two plant extracts using various toxicological investigations, as per the regulatory test guidelines, were evaluated. The mutagenic, cytotoxic, and genotoxic potential of these two plants were assayed using Ames test, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, comet assay, and micronucleus test in the bone marrow cells. **Results:** The results demonstrated that the tested doses of M.S (Roxb.) ex Link leaves extract showed mutagenic, cytotoxic, and genotoxic effects, whereas O.I (Linn.) Vent stem bark extracts showed nonmutagenic, noncytotoxic, and nongenotoxic effects. **Conclusion:** The stem bark extracts of O.I (Linn.) Vent has no mutagenic, cytotoxic, and genotoxic or clastogenic effects in our experimental conditions. However, M.S (Roxb.) ex Link leaves extract caused a significant increase in DNA damage as compared with the positive control, i.e., cyclophosphamide. Thus, the present study revealed that M.S (Roxb.) ex Link leaves extract is toxic, while O.I (Linn.) Vent stem bark extract was found to be safe.

Key words: Cytotoxicity, genotoxicity, *Meyna spinosa*, mutagenicity, *Oroxylum indicum*

SUMMARY

- For the first time, we reported the safety performance of these two plants.
- The absence of toxicity in *Oroxylum indicum* (O.I) plant extracts was observed at various doses in animals.
- Interestingly, our result indicated that *Meyna spinosa* (M.S) extract shows toxicological effect.
- Therefore, O.I plant extracts was considered as safer plant extract as compared to M.S.



Abbreviations used: MS: *Meyna spinosa*; OI: *Oroxylum indicum*.

Correspondence:

Dr. Pronobesh Chattopadhyaya,
Division of Pharmaceutical Technology,
Defence Research Laboratory, Tezpur, Assam, India.
E-mail: chattopadhyay.drl@gmail.com

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INTRODUCTION

Oroxylum indicum (O.I) (Linn.) Vent, belonging to the family *Bignoniaceae*, is a medium-sized deciduous evergreen tree up to 12 m high with light grayish brown, soft, spongy bark; large pinnate, bipinnate or tripinnate, ovate or elliptic leaves; lurid, purple, fleshy, flowers, and large, flat, sword-shaped capsules full of many flat and papery thin seeds with broad silvery wings.[1] It is distributed throughout the areas of Bangladesh, India, Malacca, Sri Lanka, Malay Islands, and China.[2] The plant is known as Sona, Sonpatti, Shoyanka, Sonpatha Kanak, or Midnight Horror in native language, which has been reported for its medicinal properties. Phytochemical studies reported that O.I bark contains large amount of flavonoids, alkaloids, glycosides, sitosterol, p-coumaric acid, and naphthalene compounds.[3] Reports indicate the plant being used as an important constituent of several Ayurvedic and tribal medicines.[4] Traditionally, the plant is used as diaphoretic, astringent, carminative, diuretic, stomachic, aphrodisiac and has high potential for stimulating digestion, curing fevers, coughs, and other respiratory disorders.[5] The plant is also reported to possess anti-inflammatory, antibacterial, antiarthritic, antifungal, antiulcer, antioxidant, diuretic, hepatoprotective, and immunomodulatory activities.[4-8] The medicinal uses of O.I and the components of the tree which encourage these uses are impressively set out in the introduction to genetic diversity in O.I.[9] The antimicrobial activity of the petroleum ether, ethyl acetate, and methanol extracts has

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been reported.[10] Numerous Ayurvedic formulations with this plant are being used as antihelminthic, antibronchitic, antirheumatic, antianorexic and for treatment of leprosy and tuberculosis.[11]

Meyna spinosa (M.S) (Roxb.) ex Link, *Rubiaceae*, is a spiny, usually a shrub or armed small tree, which can grow up to 8 m. Branches are busy, and spines are axillary, straight, sharp, 5–40 mm. Leaves are membranous, ovate or elliptic-oblong, while flowers crowded into fascicales and have shorter pedicels and petioles. Fruits are yellowish, subglobose drupe, smooth with persistent calyx lobes. The plant is used in traditional folk medicines and widely distributed in the Northeastern, Eastern, and Southern parts of India.[12] Fruits and the bark of the plant are used to treat headache,[13] while the fruits and leaves are beneficial in diabetes, jaundice, and other gastrointestinal disorders.[14,15] Tender leaves, ripe fruits, and seeds are useful to cure skin infections and pimples,[15-17] the leaf is also prescribed in indigestion and to treat dyspepsia,[18] Fruits are a good source of nutrient and are used to cure cough and as a refrigerant traditionally.[13,18] The plant is also important for its abortifacient activity; seeds and fruits are used by several ethnic groups in India to induce abortion.[19] Recently, two compounds were isolated from the fruits of M.S which possess antimicrobial activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Candida*. One was identified as oleanolic acid which possesses the highest antimicrobial activity.Buragohain and Goswami *et al.*[16,20] have also reported the antifungal activity of the methanol extract of M.S. Thus, the interest in herbal medicines used for the prevention and therapeutic treatment of diseases has increased due to the increasing resistance of microorganisms to synthetic antibiotics, raising costs, and side effects of synthetic drugs for the maintenance of personal health. Similarly, natural products have been traditionally used as conventional remedies due to popular belief that they possess negligible side effects.[21] However, several natural compounds have been reported to act as mutagens and/or carcinogens.[22-24] These genotoxic insults often lead to cancer,[25] Alzheimer's disease,[26] and other chronic degenerative diseases, such as atherosclerosis and other cardiovascular diseases, which are the leading cause of death in human and animal populations.[27] As such, genotoxicity studies of both naturally occurring and synthetic substances are of great interest because of the widespread and often chronic use of specific or fictitious herbal remedies, modern medicinal products, and food ingredients, as well as other household and environmental chemicals.[28] Moreover, governmental regulatory policies worldwide are now making it vital that all newly produced natural or synthetic substances, with or without antitumor properties, be subjected to genotoxic/mutagenic screening.[29]

Hence, recent concerns have been raised over the lack of quality control and scientific evidence for the safety of herbal medicine.[30] However, finger count scientific investigations have explored the safety and toxicity of herbal medicines.[31-33] However, to the best of our knowledge, no investigations have been performed till date based on these two plants in terms of their safety and toxic profile. Considering the applauded medicinal significance of these two plants and the need for practical data on safety, we assessed the mutagenicity, cytotoxicity, and genotoxicity studies employing *in vitro* and *in vivo* experimental models. Thus, our research objectives directed us toward the initiation of this study for the first time to explore the possible toxic effect of these two plants in accordance with the Organization for Economic Cooperation and Development (OECD) test guidelines.

MATERIALS AND METHODS

Collection of plant materials

The fresh, mature whole leaves of M.S and stem bark of O.I were collected from Tezpur, Assam, during April 2012. The plant was identified by its vernacular name and later validated by Prof. (Dr.) S. K. Borthakur, Department of Botany, Gauhati University, Guwahati, Assam, India. The identified voucher specimens of O.I (GUBH 3964) and M.S (GUBH 3965) were deposited at the Gauhati University Botany Herbarium, Department of Botany, Gauhati University, Guwahati, Assam, India, for future reference.

Preparation of the extracts

The plant parts were washed thoroughly under running water, cut into smaller pieces, and then air-dried. The air-dried parts were grinded with a mechanical grinder into coarse powder. The powdered materials were extracted with methanol using a Soxhlet apparatus for solvent extraction, which was concentrated to dryness under reduced pressure to yield hydroalcoholic mixture (50:50% v/v). The mixtures were then filtered by filter paper. The extracts were then kept in Petri dish under water bath at a temperature of 50°C –55°C until evaporated to dryness. The dried extracts, herein referred to as M.S and O.I extracts, were then collected into a glass container and stored in a desicator.

Mutagenicity

Ames test

All the plant extracts were examined for its mutagenic potency in four histidine-requiring *Salmonella typhimurium*-mutant strains. *S. typhimurium* strains were obtained from Institute of Microbial Technology, Chandigarh, India. The strains used were TA98 and TA1538 which detect frameshift mutations, TA100 and TA1535 which detect basepair substitutions.[22,34] This assay was performed according to the plate incorporation procedure described by the OECD test guideline 471 recommendations. [35,36] Tester bacteria were exposed to four different concentrations ranging from 2.5 up to 5 μg/plate, with and without metabolic activation. Three parallel plates were tested in each concentration. Negative and positive controls were run simultaneously with the test.

Animals and dosing

Healthy, adult Balb/c albino mice (weighing 20–25 g, 5–6 week age, female) were obtained from Central Animal Resources, Defence Research Laboratory, Tezpur, Assam, India. The animals were placed in polypropylene cages, with free access to standard laboratory diet (Pranav Agro Industries Limited, Maharashtra, India) and provided water *ad libitum*. Animals were housed in an environmentally controlled room with temperature of 22°C ± 3°C and 40%–70% relative humidity with a 12-h light/dark cycle for an acclimation period of 7 days to laboratory conditions before the beginning of the experiment. All the experimental procedures described were performed according to the "Principles of Laboratory Animal Care" and approved by the Institutional Animal's Ethical Committee. The Balb/c albino mice were divided into four groups, i.e., control (C), positive control (cyclophosphamide [CP]; 50 mg/kg bw), M.S-treated group (400, 800 and 1600 mg/kg) and O.I-treated group (500, 1000, and 2000 mg/kg) by gavage for 14 days. All the mice were then sacrificed after the treatments by cervical dislocation and the cells were prepared for further analysis.

Cytotoxicity study

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The effect of M.S and O.I on hepatocyte primary cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[37] The hepatocytes were seeded in 96-well plates. Each treated cells was added to the medium. A vehicle control was included in which cells were incubated only with solvent (0.1 M $NaHCO_3$). After 12 h treatment, cells were washed with PBS and a fixed concentration of MTT in PBS was added to each microwell. After incubation for 4 h at

37°C, the supernatant was removed, the insoluble crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm by using a Microplate Reader (SpectraMax Plus384; Molecular Devices, Sunnyvale, CA, USA).

Measurement of apoptosis by flow cytometer

Cell apoptosis was evaluated by a flow cytometer (Guava easyCyte 6HT; Merck Millipore, Darmstadt, Germany). The cells from each treated groups were centrifuged at 50 ×*g* at 4°C for 5 min, the supernatant was discarded, and the pellets were washed with 1× phosphate-buffered saline (PBS) three times and finally resuspended in 50 µl of 1× PBS. From this suspension, cells per ml were mixed with the Guava Nexin reagent (Merck Millipore) following the manufacturer's instruction. The cells were analyzed using Guava software version 2.2.[38]

Genotoxicity study

Comet assay

The comet assay was performed for *in vivo* genotoxicity evaluations.[39] The blood samples were collected from the retro-orbital plexus after treatment and before euthanasia and treated with 1× red blood cell lysis buffer for 10 min at 25°C and leukocytes were isolated and suspended in 50 µl of PBS (pH 7.5). Cells were mixed with 100 mL of 0.5% low melting point agarose at 37°C and rapidly spread onto microscope slides, precoated with 1% normal melting point agarose. The slides were coverslipped and allowed to polymerize at 4°C for 20 min. The coverslips were gently removed and the slides were then immersed in cold, freshly prepared lysing solution for consisting of 44.5 mL of a stock solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH set to 10.0 with ~8 g solid NaOH, 890 mL of distilled water, and 1% sodium lauryl sarcosine), plus 0.5 mL of Triton X-100 and 5.0 mL of DMSO. The slides were allowed to stand at 4°C for 1 h and then placed in a high pH (>13) electrophorosis buffer (300 mM NaOH, 1 mM Na_2-EDTA, pH 13.0) at 4°C for 20 min before electrophoresis, to allow DNA unwinding. The electrophoresis run was performed at 4°C under dim light at 300 mA and 25 V for 30 min. The slides were then submerged in a neutralization buffer (0.4 M Tris–HCl, pH 7.5) for 15 min and stained with 20 µg/mL 4',6-diamidino-2-phenylindole for 10 min and cover slipped. The material was evaluated immediately at a fixed magnification, using a fluorescence microscope (Coslab HL-23; Cos Lab, Haryana, India). A total of 100 nonoverlapping comets per sample on each randomly coded slide were scored using TriTek Comet Score™ (Sumerduck, VA, USA). The cells were classified into four different comet score classes: class 0 - undamaged cells; Class I - tail shorter than the diameter of the head (nucleus); Class II - tail length 1–2 times the diameter of the head; and Class III - tail length more than twice the diameter of the head. The cells were blindly scored using light microscope at a higher magnification.

Micronucleus assay

The micronucleus (MN) test was conducted in accordance with the OECD guideline 474[35] and the protocols were followed as recommended.[40,41] The bone marrow from one femur was flushed out using 2 mL of saline (0.9% NaCl) and centrifuged for 10 min. The supernatant was discarded and smears were made on slides. The slides were coded for a blind analysis, fixed with methanol, and stained with 5% Giemsa[42] For the analysis of the micronucleated polychromatic erythrocytes (MNPCE) per treatment group observed in bone marrow cells of mice were scored to determine the clastogenic property of the extract. The cells were blindly scored using a light microscope at higher magnification. The mean number of MNPCE in individual mice was used as the experimental unit, with variability (standard deviation) based on differences within the same group.[43,44]

Statistical analysis

Data were expressed as mean ± standard error of the mean. The data obtained from the comet assay were subjected to analysis of variance (ANOVA) and analyzed by Tukey's Test (significant level at $P < 0.05$). For genotoxicity study, the distribution of the comet figures did not follow a Gaussian distribution. The data obtained from the MN assay were submitted to the ANOVA test with linear regression, both using the GraphPad InStat software (version 3.01, California corporation USA). The results were considered statistically significant at $P < 0.05$.

RESULTS

Mutagenicity

Ames test

A serious concentration-dependent increase in M.S and no increase in the number of revertant colonies occurred in the four test strains (TA-98, 100, 1538, and 1535) at any concentrations of O.I, either in the presence or absence of S9 mixture [Table 1], and similar findings were obtained from the spot test results [Figure 1]. No obvious concentration-depended relationship had been found only in the case of O.I extract; therefore, the Ames test result of O.I extract was negative. However, for M.S extract, the test result was shown to be positive.

Cytotoxicity study

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

MTT assay was performed to determine cell viability by measuring the conversion of tetrazolium salts to formazan, the amount of which is proportional to the number of living cells [Figure 2]. The viability of cells was not affected by the control, vehicle, and O.I-treated group (>90%). However, a significant decreased on cell viability was observed in M.S-treated group in a dose-dependent manner (<65%; $P < 0.05$).

Measurement of apoptosis by flow cytometer

Flow cytometric analysis of hepatocyte cells using Guava Nexin reagent (stained with Annexin V-PE and 7-AAD) indicates M.S at a concentration of 400 mg/kg body weight induced cellular apoptotic events in hepatocyte primary cell culture. The results indicated that control, vehicle-treated, and O.I at a concentration of 2000 mg/kg body weight treated cells contained approximately 94%, 89%, and 85% viable cells, 4%, 6%, and 8% early apoptosis, 2%, 3%, and 4% late apoptosis, and 0%, 2%, and 3% necrosis, respectively. On the other hand, M.S treatment at different concentrations resulted in approximately 51%–61% viable cells, 13%–16% early apoptosis, 14%–19% late apoptosis, and 9%–17% necrotic cells [Figure 3]. These findings clearly indicated that cell viability is decreased and apoptotic events (both early and late stages) are increased with M.S-treated group in a concentration-dependent manner which ultimately revealed the cytotoxic nature of M.S at concentration of 400 mg/kg body weight and noncytotoxic nature of O.I extracts at concentration of 2000 mg/kg body weight [Figure 4].

Genotoxicity study

Comet assay

The comet assay indicated that DNA damage did not occur in the control, vehicle, and at all concentrations of O.I-treated groups. On the other hand, significant DNA damage was observed in M.S-treated group in a concentration-dependent manner in comparison to an untreated control group, as evidenced from the comet assay results and score, i.e., tail length, %DNA in tail, and olive moment [Table 2]. Furthermore, the cells treated with positive control (50 µg/ml, CP) exhibited a higher DNA damage index ($P < 0.05$) as compared to the others except M.S-treated group [Figure 5].

Table 1: Ames test results of *Oroxylum indicum* and *Meyna spinosa* extracts in four strains of *Salmonella typhimurium*

Mutant strain	Dose level (µg/plate)	O.I		M.S	
		Revertant colonies (mean±SD)		Revertant colonies (mean±SD)	
		Without activation	With activation	Without activation	With activation
TA-98	0	24±1.3	31±3.7	28±2.0	27±2.8
	0.625	21±2.9	27±2.3	80±3.3	76±2.4
	1.25	19±3.2	25±3.9	141±1.1	150±3.0
	2.5	26±3.6	23±3.2	208±2.2	234±4.2
	5	29±2.9	33±2.7	274±3.1	322±2.7
	4-NPD*	431±7.9	367±9.1	417±7.7	439±9.4
TA-100	0	28±0.9	17±3.4	20±2.5	35±3.3
	0.625	31±1.6	26±3.1	72±3.7	81±2.1
	1.25	21±3.9	24±2.3	128±3.1	173±2.7
	2.5	25±2.4	31±4.2	223±1.4	271±3.2
	5	24±2.2	19±3.9	296±3.6	324±3.1
	SA**	395±11.9	389±12.4	378±10.5	421±11.4
TA-1538	0	19±3.3	26±3.2	35±1.8	32±3.3
	0.625	27±2.9	25±2.1	89±3.9	55±2.1
	1.25	22±1.5	31±2.3	144±2.1	121±2.3
	2.5	26±1.9	24±3.2	202±3.9	262±2.5
	5	33±2.1	31±2.0	332±1.5	349±2.2
	4-NPD*	377±8.1	423±9.2	352±12.9	418±10.2
TA-1535	0	23±1.8	34±2.2	19±4.0	25±2.2
	0.625	34±4.1	24±1.9	85±2.9	76±3.3
	1.25	29±3.1	21±3.9	138±3.3	114±3.1
	2.5	24±2.5	32±1.5	261±3.8	234±2.9
	5	22±0.6	22±3.9	326±3.1	291±2.1
	SA**	441±8.1	433±12.5	381±11.6	369±9.5

**SA for TA 100 and TA 1535; *4-NPD for TA 98 and TA1538 strains. O.I: *Oroxylum indicum*; M.S: *Meyna spinosa*; SD: Standard deviation; SA: Sodium azide; 4-NPD: 4-Nitro-ophenylenediamine

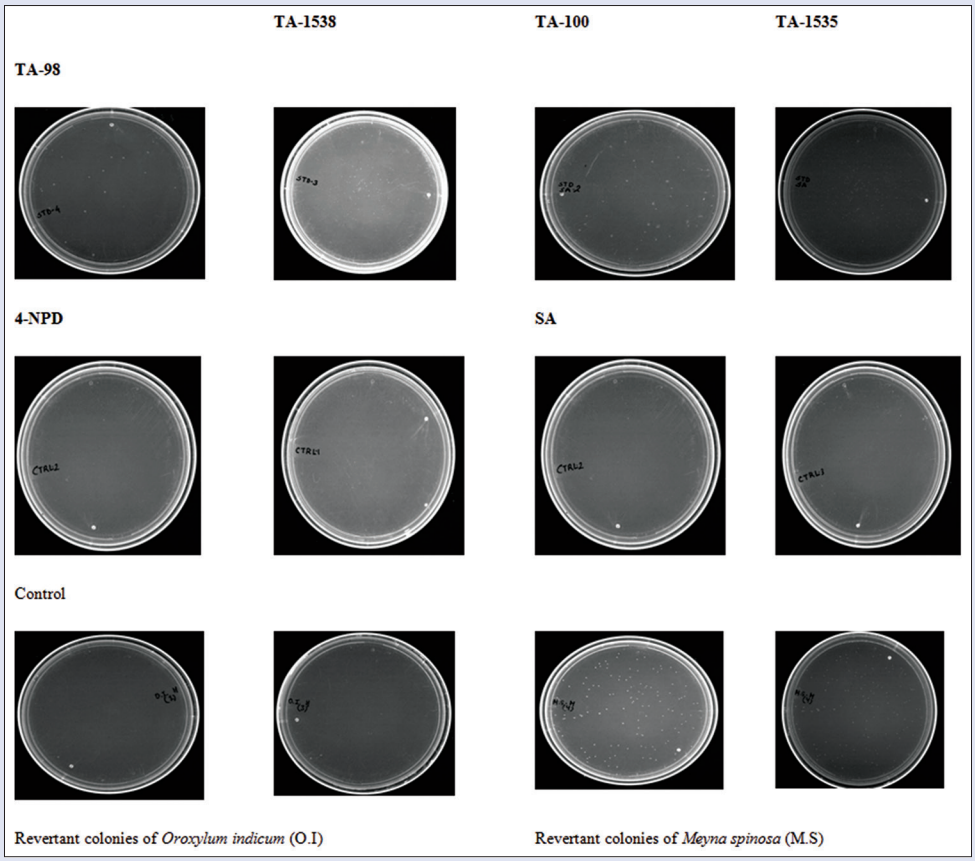

Figure 1: Spot test with different *Salmonella typhimurium* strain

Micronucleus assay

The MN test is based on the evaluation of an increase in the frequency of polychromatic erythrocytes with micronuclei. In our study, the results of clastogenic and anticlastogenic analysis obtained for the treatment are provided in Table 3. Statistically significant differences ($P < 0.05$) were exhibited in the frequency of MNPCEs between the control and M.S-treated group, indicating a presence of clastogenic/aneugenic effects induced by plant extracts [Figure 6]. However, animals treated with positive control (CP) showed a high frequency of MNPCE in the bone marrow cells when compared to the control ($P < 0.05$). However, the frequencies of MNPCE in the MN test in mice were not significantly statistically different between O.I-treated group and control group.

In this study, the results showed that O.I-treated group did not induced any toxicological signs, thus reveals its safety nature. However, surprisingly, M.S altered the toxicological profiles and revealed toxic in nature. As expected, the positive controls (CP) showed significant increases in the frequency of necrotic cells when compared with the control.

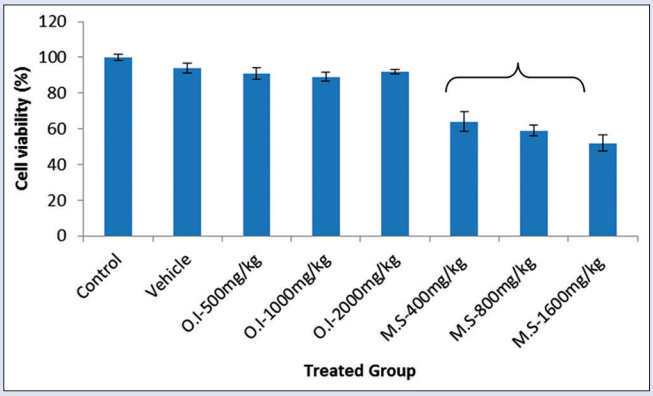

Figure 2: Results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on percentage cell viability. Data are expressed as mean ± standard error of the mean ($n = 6$). Asterisk (*) indicates statistical significance ($P < 0.05$)

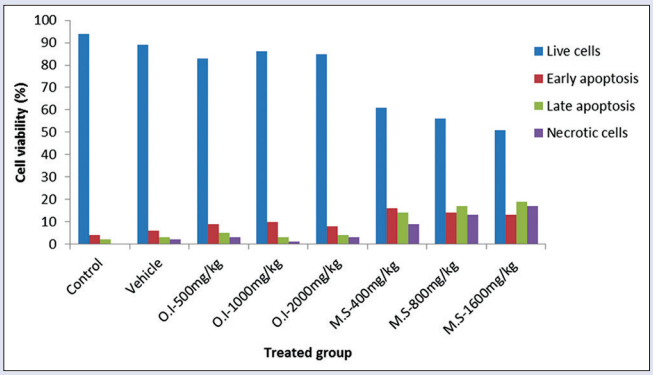

Figure 3: Flowcytometric evaluation of *Oroxylum indicum* and *Meyna spinosa* extracts at different concentrations in apoptotic events on cell viability (%) by primary cell culture using Guava Nexin assay kit

DISCUSSION

A series of safety studies were performed systematically to investigate the safety of the stem bark extract of O.I and M.S leaves extract in Northeast region. The results of the current study are consistent by using the tester strains TA98, TA100, TA1535, and TA1538 to evaluate the mutagenicity of extracts by the Ames assay. Mutagenicity was induced only by M.S extracts in a concentration-dependent manner with and without metabolic activation in *S. typhimurium* tester strains. In the present study, tester strains TA98 and TA1538 were used to detect frameshift mutations, whereas tester strains TA100 and TA1535 were used to detect basepair substitution mutations. Different investigators have used different tester strains to determine the mutagenicity of test materials.[45] It is noted that some biomaterials are mutagenic to one tester strain while it is not mutagenic to another. Even though many investigators have sometimes used just two strains to determine the mutagenic potential of materials, it is felt that the use of at least four tester strains as recommended by Mortelmans and Zeiger[46] gives a more definite result.

Cytotoxicity is an important parameter for assessing chemical agents for toxicity and health risks. In genotoxicity testing, cytotoxicity analysis is a prior step because the cytotoxic effect of chemicals may lead to false interpretation of genotoxicity. In the present study, we observed that hepatocytes in the control, vehicle, and O.I-treated groups at a various dose showed no significant cytotoxic effect (>90% viable cells). However, it was also interesting to note that M.S-treated groups showed a potent cytotoxic effect in a concentration-dependent fashion (<65%; $P < 0.05$) in hepatocytes. Again, the results of flow cytometric analysis of apoptosis correlated with the morphological studies showed more precisely that

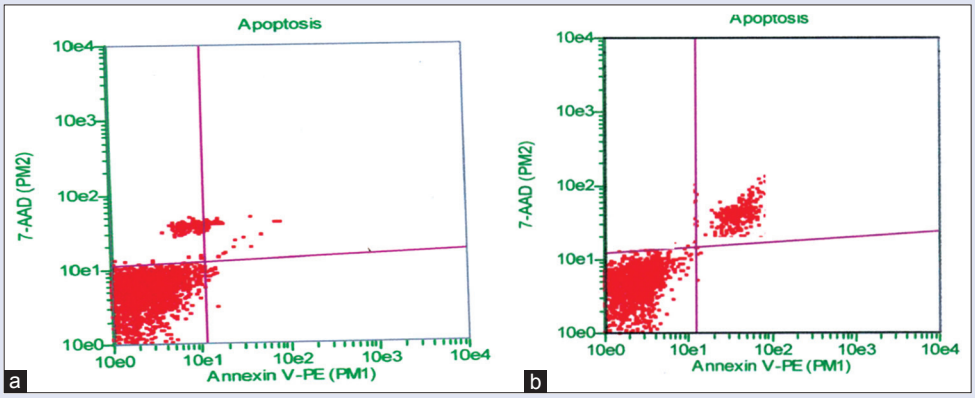

Figure 4: Flowcytometric analysis of apoptosis in blood cells by staining with Annexin V-PE (PM1) and 7-AAD (PM2): (a) *Oroxylum indicum* (2000 mg/kg body weight) treated lower left quadrant, viable cells (86.09%), lower right quadrant, early apoptosis (7.93%) and upper right, late apoptosis (3.70%) and (b) *Meyna spinosa* (400 mg/kg body weight) treated lower left quadrant, viable cells (61.23%), lower right quadrant, early apoptosis (16.11%) and upper right, late apoptosis (13.88%)

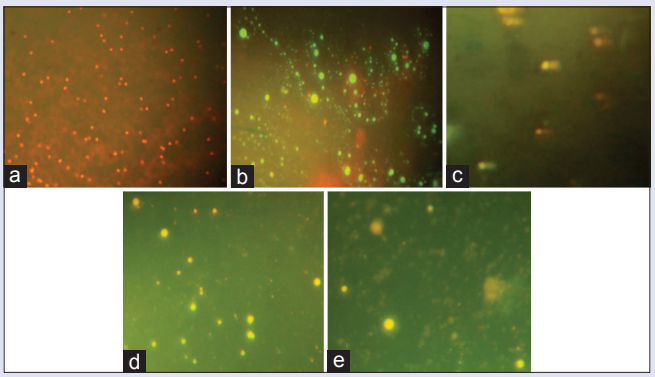

Figure 5: Comet assay images of (a) control, (b) vehicle, (c) positive control (50 µg/ml cyclophosphamide), (d) *Oroxylum indicum* (2000 mg/kg body weight) treated and (e) *Meyna spinosa* (400 mg/kg body weight) treatment for the assessment of genotoxicity in hepatocyte primary cell culture (×100)

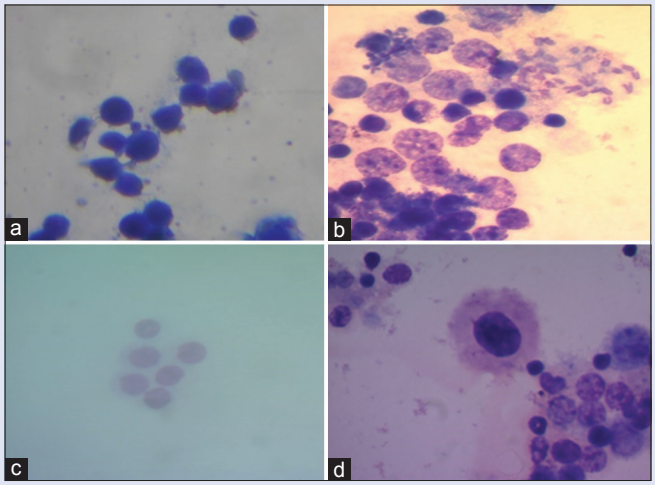

Figure 6: Micronucleus images of (a) control, (b) positive control (50 µg/ml cyclophosphamide), (c) *Oroxylum indicum*. (2000 mg/kg body weight) treated and (d) *Meyna spinosa* (400 mg/kg body weight) treatment for the assessment of genotoxicity in bone marrow cells of mice (×100)

Table 2: Comet assay for the assessment of genotoxicity of *Oroxylum indicum* and *Meyna spinosa* extracts in hepatocyte primary cell culture

Treatment	Tail length	% DNA in tail	Olive moment	Score
Control	0.79	0.08	0.49	0
Vehicle	0.74	0.20	0.53	I
Positive control-CP	9.56*	3.21*	8.72*	III
O.I-500 mg/kg	0.78	0.33	0.52	I
O.I-1000 mg/kg	0.72	0.25	0.66	I
O.I-2000 mg/kg	0.76	0.27	0.61	I
M.S-400 mg/kg	3.98*	2.79*	1.88*	II
M.S-800 mg/kg	5.57*	3.6*	2.45*	III
M.S-1600 mg/kg	7.72*	4.89*	3.08*	III

*Considered significant ($P<0.05$). O.I: *Oroxylum indicum*; M.S: *Meyna spinosa*; CP: Cyclophosphamide

Table 3: Percent of micronucleated polychromatic erythrocytes observed in bone marrow cells of mice for the assessment of genotoxicity of *Oroxylum indicum* and *Meyna spinosa* extracts

Treatment	MNPCE (%)	Remarks
Control	0.19±0.08	MNPCE
Positive control-CP	14.62±2.08*	Clastogenic
O.I-500 mg/kg	0.26±0.11	MNPCE
O.I-1000 mg/kg	0.31±0.17	MNPCE
O.I-2000 mg/kg	0.28±0.23	MNPCE
M.S-400 mg/kg	5.62±1.25*	Clastogenic
M.S-800 mg/kg	7.31±2.19*	Clastogenic
M.S-1600 mg/kg	8.44±1.23*	Clastogenic

*Considered significant ($P<0.05$). MNPCE: Micronucleated polychromatic erythrocytes; O.I: *Oroxylum indicum*; M.S: *Meyna spinosa*; CP: Cyclophosphamide

the frequency of early apoptotic cells reached a peak level. The frequency of late apoptotic cells gradually increased and reached a maximum level after a certain period of M.S treatment at highest dose (1600 mg/kg body weight). We compared the comet assay results (the significantly increased level of DNA single strand breaks) with flow cytometric data (the significantly increased frequency of late apoptotic cells, a possible confounding factor) and observed similar correlation. Hence, it may be expected that M.S-treated group leads to break DNA single strand that are related to genotoxicity and cytotoxicity as well. However, the molecular mechanism behind cellular apoptosis is still not clear and needs to be investigated further.

Since M.S extract reveals cytotoxic which is highly toxic in nature, thus, the aim of the present study was to investigate the DNA damaging properties of M.S. Therefore, the present study evaluated the genotoxic effect of M.S extract (cytotoxic and noncytotoxic) to draw conclusions about its relevance in cellular toxicity using the established comet assay. The comet assay has become one of the standard methods for assessing DNA damage due to its simplicity, sensitivity, versatility, speed, and economy. The alkaline (pH >13) assay detects single-strand breaks, cross-links, incomplete excision repair sites, as well as apurinic or apyrimidinic sites, which are alkali labile.[46] Finally, in our study, no significant increase in the total comet score was detected between the control, vehicle, and O.I-treated group, but M.S elevated the comet score at elevated doses. This might be due to the excessive production of reactive oxygen species, which induce oxidative stress and might lead to lipid peroxidation. Therefore, the present findings suggested an indirect genotoxic effect in which the oxidative stress preceded cytotoxicity and DNA damage, leading to carcinogenicity.

CONCLUSION

Herbal medicine is widely popular as a primary therapeutics or supplements for improving health related problems. However, scientific evidence for the safety of the herbal products has become an important concern and requires regulatory clearance for wide acceptance. Although many studies have been reported in context to the extensive investigations of these two plants properties based on their efficacy evaluations, in molecular level toxicological investigations in terms of mutagenicity, cytotoxicity and genotoxicity and/or antigenotoxicity have not been investigated properly so far. Therefore, for the first time, we evaluated the safety performance of these two plants using standard toxicological tests recommended as per the OECD test guidelines and our test result indicated the absence of toxicity in O.I plant extracts at various doses. Interestingly, our result indicated that M.S extract shows toxicological effect which is completely concentration dependent in comparison to O.I extract. Thus, our research objective motivated us just to drop this plant extract for further efficacy assessment study but directed us toward the exploration of O.I plant extract for efficacy assessment against mycotoxin-induced toxicity in experimental animal model. Moreover, further investigations are also needed to clarify the

protective mechanism of O.I extract against mycotoxin-induced toxicity, which may be of great pharmacological importance, and might be beneficial for cancer prevention.

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Conflicts of interest

There are no conflicts of interest.

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