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Evaluation of genotoxic effects of methanolic extract of brown seaweed *Stoechospermum marginatum*

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ABSTRACT

Seaweeds are considered as a valuable source of bioactive compounds produced as secondary metabolites having a broad spectrum of biological activities. Recently seaweed has been extensively utilized by pharmaceutical, chemical and nutraceutical industries as valuable sources of raw material of natural origin. The present study aimed at evaluating the mutagenic and genotoxic effects of methanolic extract of brown seaweed *Stoechospermum marginatum*. The test extract was assessed for its potential mutagenic effect using *in vitro* bacteria reverse mutation assay (Ames test) and for its potential clastogenic and DNA damaging effects using *in vivo* rodent bone marrow micronucleus and alkaline comet assay respectively. The test extract did not produce a two-fold increase or a reproducible dose-dependent increase in the number of revertant colonies in all the bacterial tester strains at the highest dose of 5000 µg/plate tested both with and without metabolic activation. No significant clastogenic and DNA damaging effects were observed at the highest dose of 2000 mg/kg body weight when the test extract was assayed *in-vivo* in mice bone marrow erythrocytes and peripherally in lymphocytes respectively. The result obtained from this study revealed that methanolic extract of *Stoechospermum marginatum* is safe to living cells genome and does not produce significant mutagenic and genotoxic effect when assayed in both *in vitro* and *in vivo* testing systems.

Keywords: Ames test, mutagenic, clastogenic, genotoxic, micronucleus.

INTRODUCTION

The exploitation of plants natural source of medicine has been in practice since ancient time. Today, advancement in science and technology has provided a better means of exploring plant materials with excellent therapeutic potentials serving as a lead for the development of novel drugs [1].

Recently several studies have revealed that some natural products that are being consumed as food or used in traditional medicine were found to be cytotoxic, mutagenic and genotoxic. This necessitates the evaluation of toxicity profile of compounds of plant origin with potential therapeutic applications [2]. Mutagens are chemical substances that have the ability to induce a permanent change in the amount or structure of genetic material of an organism leading to mutation which usually associated with the potential of inducing cancer [3]. Evaluation of genotoxic effect of a compound is an important component of the safety assessment of chemical substances, including pharmaceuticals, agricultural chemicals, food, additives and industrial chemicals [4].

Seaweeds contain highly bioactive secondary metabolites that served as a useful drug leads towards the development of new pharmaceutical agents. Recently, compounds from seaweeds have generated an enormous amount of interest to pharmaceutical and nutraceutical industries as valuable source of bioactive compounds with immense medicinal and industrial applications [5]. The east coast of India is a unique marine habitat with diverse population of seaweeds, Indian seaweeds are less consumed as food like used to be in other far east Asian countries but mainly exploited as a source of phycocolloids such as agar-agar, agar alginate and carrageenan used by pharmaceuticals, chemical as well as food industries mostly as gelling, stabilizing and thickening agents [6].

Stoechospermum marginatum is a marine macroalgae belonging to the class phaeophyceae (brown algae) of family Dictyotaceae and being one of the most important renewable resources abundantly present in the Indian marine environment [7]. *S. marginatum* was found to be rich in active phytoconstituents such as alkaloids, flavonoid, tannin, saponins, phenols, sterols, sulfated fucans, agar and alginic acid [8].

These active phytoconstituents were found to have numerous health-promoting effects including anti-oxidative, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects [9].

In the present day context of increased demand for utilization of compounds of natural origin, it is very much essential to carryout studies on the genotoxicity profile of abundantly available plants resources with huge medicinal values such as seaweeds. Considering its importance of usage as pharmaceutical and nutraceutical agent, the present study was undertaken with the objective to investigate the potential mutagenic and genotoxic effects of methanolic extract of *S. marginatum* using *in vitro* Ames test and *in vivo* rodent bone marrow micronucleus and alkaline comet assay.

MATERIALS AND METHOD

Collection and processing of extract

Fresh samples of plant (*Stoechospermum marginatum*) were collected in clean polythene bags containing sea water from the coastal line of Mandapam, Ramanathapuram district, Tamil Nadu, India (latitude 9°18'N and longitude 79°6'E) and immediately transported to our Departmental Laboratory for processing. A small portion of the algal plant was sent to Botanical Survey of India (BSI), Coimbatore for identification, it was confirmed as true species and a voucher specimen was deposited there. The algal materials were washed thoroughly under running tap water for removing the attached epiphytes and other marine debris and allowed to dry under shade for 5–7 days, until the moisture was completely removed. The dried material was ground to powdered form and stored in air tight containers at room temperature. Crude extract of *S. marginatum* was obtained using absolute methanol according to the methods described by [6] with some modifications.

Extraction was carried out by soaking 10g of the powdered sample in 100 ml of the solvent (1:10 – w/v) in a conical flask and kept on a shaker (120 rpm) at room temperature for 48hrs. The mixture was filtered using Whatman™ No. I filter paper and the process was repeated until the solvent becomes colorless. The filtrates obtained were combined and concentrated by evaporating the solvent under reduced pressure at its boiling point using rotary evaporator. The solvent was totally removed and the dried extract obtained was weighed and stored at 4°C until further use. The dried extract yield was approximately 4.5%.

Chemicals

Ampicillin powder (Sigma-Aldrich), Tetracycline powder (Sigma-Aldrich), Fetal calf serum (Himedia), Dimethyl sulfoxide (DMSO) (Loba chemie), Cyclophosphamide (Sigma-Aldrich), Ethidium bromide (Sigma-Aldrich), Sodium azide (Sigma-Aldrich), Mitomycin C (Sigma-Aldrich), 2-Nitrofluorine (2-NF) (Sigma-Aldrich), 9-Aminoacridine (9-AA) (Sigma-Aldrich), Benzo[a]pyrene (B[a]P) (Sigma-Aldrich), β -nicotinamide adenine dinucleotide Phosphate (NADP) (SRL), D-Glucose-phosphate (SRL), L-Histidine HCL (SRL), D-Biotin (SRL), Low melting point (LMP) agarose (SRL), Normal melting point (NMP) agarose (SRL), Crystal Violet (Himedia), Dextrose (Himedia), Bactor agar (SRL), Oxoid Nutrient broth No. 2 (Himedia), Potassium phosphate Di-basic anhydrous (Loba Chemie), Potassium chloride (Himedia), Magnesium chloride (Himedia) were all utilized in the studies. All other reagents and chemical used were of analytical grade.

Bacterial tester strains

Five histidine dependent (*His*⁻) tester strains of *Salmonella typhimurium* viz., TA98, TA100, TA102, and TA1535 and TA1575 were procured from GRL Laboratories private limited, Chennai, Tamil Nadu and used in this study. The identity of the tester strains was confirmed for the following genotypic characteristics viz., histidine and biotin requirement, crystal violet sensitivity, ultra violet sensitivity, ampicillin and tetracycline resistance as described by [3] before conducting the assay.

Preparation of S9 mixture

S9 mixture was prepared according to the method recommended by [3]. The components of S9 mixture were, 8 mM magnesium chloride, 33 mM potassium chloride, 5 mM G-6P, 4 mM NADP, 100 mM sodium phosphate (pH 7.4) and 10% S9 fraction (v/v). A positive mutagen, B[a]P was used to check the quality of S9 mixture.

Limit Dose / Cytotoxicity study

A preliminary limit dose and cytotoxicity study of methanolic extract of *S. marginatum* (500, 1500, and 5000 μ g/plate) dissolved in DMSO was done with *Salmonella typhimurium* strain TA98. Overnight grown culture of TA98 was mixed with 0.1 ml of each concentration and plated and grown on nutrient agar plates, as described by [10]. A test concentration with >50% viable cells was considered non-toxic compared with the viability of the negative (solvent) control. All the concentrations tested showed no sign of cytotoxicity and growth inhibition in the tester strain used. The test extract did not produce precipitation or discoloration of the plated medium

Mutagenicity assay

The bacterial reverse mutation assay was conducted using plate incorporation method both with and without metabolic activation as described by [3, 11]. 2-NF, NaN₃, MMC, and 9-AA were used as direct acting positive mutagens while B[a]P was used as indirect acting mutagen. The test extract and the corresponding positive control mutagens were dissolved in DMSO as a vehicle. Five different concentrations of the test extract viz., 312.5, 625, 1250, 2500 and 5000 μ g/plate were chosen based on a preliminary dose range finding and cytotoxicity study for conducting the test in all tester strains. Briefly, for each treatment, 0.1 ml of the test extract (for all five doses) or control preparation was introduced into a sterilized test tube, to which 0.1 ml of overnight grown bacterial culture was added. For preparations with S9 mix, 0.5 ml was also added; for preparations without S9 mix, 0.5 ml of 0.1 M sodium phosphate buffer solution was added. After a gentle mixing, 2ml of molten top agar (45°C) containing 0.5 mm histidine/biotin was added. The mixture was then vortexed and poured on to minimal glucose agar plates. After the top agar solidified, the plates were inverted and incubated for 48hrs at 37°C. Following incubation, plates were examined for precipitation and toxicity and all the experiment were performed in triplicate.

In vivo rodent bone marrow micronucleus assay

In vivo rodent bone marrow micronucleus assay was performed using Balb/c mice's bone marrow erythrocytes following guide lines and protocols described by [12, 13].

Animals

Inbred Balb/c mice (*Mus musculus*) of age 6–8 week-old weighing around 22–26 g were obtained from Laboratory Animal Medicine, TANUVAS, Madhavaram Milk Colony Chennai-51, India. The animals were housed in polypropylene cages containing certified paddy husk as bedding and were allowed to acclimatize for one week at our college Centralized Laboratory Animal House. Animals were maintained under good environmental condition with temperature and humidity of the room between 22 \pm 3°C and 50–60% respectively and under a 12 hours light/dark cycles. Commercial standard pelleted diet and wholesome water were provided *ad libitum*. All protocols used in this experiment were approved by our Institutional Animal Ethics Committee [1467/DFBS/IAEC/2018/07].

Animals treatment and sacrifice

Experiment was performed using ten animals per group. Mice were dosed orally for seven consecutive days at three different dose levels of the test extract viz., 500 mg/kg, 1000 mg/kg, and 2000 mg/kg BW based on preliminary toxicity test we performed. 1% DMSO in distilled

water was used as vehicle control. Positive control groups received single intra peritoneal (i/p) administration of CP (50 mg/kg) on the seventh day. Experiment was terminated by sacrificing five animals from each group at 24 hrs and 48 hrs of interval after the seventh day of treatment. All mice were anesthetized by isoflurane for blood sample collection by cardiac puncture and were sacrificed by cervical decapitation. All efforts were made to minimize suffering during the processes.

Bone processing and slides examination

After animals sacrifice, both side femoral bones were removed after dissection and cutting through the pelvis and tibia. The proximal end of the femur was carefully shortened until a small opening to the marrow canal became visible. Immediately femur was submerged in a 0.5 ml of chilled FCS contained in microcentrifuge tube and bone marrow was aspirated after several flushing were performed. The cells were centrifuged at 1000 rpm for 5–10 minutes and two drops of cell suspension from each animal were placed onto a clean, grease free, dry slide and smeared, fixed in methanol and stained with May-grunwald and Giemsa stain. The slides were examined using light microscope under oil immersion objective lens for the presence of micronucleus. For each animal, a minimum of 2000 polychromatic erythrocytes (PCE) were screened for determining the presence of micronuclei and the percentage of cells containing micronucleus on the total number of cells. Simultaneously, the normochromatic erythrocytes (NCE) were scanned from each animal to determine the ratio of PCE and NCE.

In vivo alkaline comet assay

In vivo alkaline comet assay performed using the whole blood collected from the treated mice following the method described by [14]. The whole blood was collected in K₂EDTA blood collection tube and equal volume of freshly prepared cold RPMI 1640 medium containing 10% DMSO was added. The mixture was transferred to 2 ml cryovials, capped and immediately frozen at -80°C. The whole was thawed in a water bath at 37°C for 1–2 min just before commencement of comet assay.

Slide processing and examination

Comet slides were prepared by dipping a grease free frosted glass slide to a coplin jar containing 1% normal melting point (NMP) agarose at 45°C up to two-third of the frosted end. The slides were removed and the undersides were wiped with clean gauze for removing the agar and were allowed to dry. 10 µl of whole blood was mixed with 75 µl of 0.5% low melting point (LMP) agarose at 37°C in an eppendorf tube and mixed gently. 80 µl of the mixture was added to the surface of pre-coated slides and a cover slip was placed. The slides were allowed to solidify at 4°C for 5 minutes. Later the cover slips were carefully removed and a third layer of 80 µl of 0.5 per cent LMP agarose was added and cover slips were placed again. The slides were allowed to solidify at 4°C for 5 minutes. The slides (without cover slips) were immersed in freshly prepared, cold lyzing solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris HCl (pH 10), 1% Triton X-100 and 10% DMSO) and refrigerated for one hour or overnight for the cell soluble component to lyzed. The slides were then removed from the lyzing solution and placed on a horizontal gel electrophoresis unit. The unit was filled with freshly prepared electrophoresis buffer (1 mM Na₂EDTA and 300 mM NaOH) to a level 0.25 cm above the slides. The slides were allowed to set in this high pH buffer for 20–40 minutes to allow unwinding of DNA before electrophoresis. Electrophoresis was carried out in the same alkaline buffer for 25 min at 25 V (0.66 V/cm) adjusted to 300 mA by raising or lowering the buffer level in the tank. Slides were then drained, placed on a tray and rinsed slowly in neutral buffer (0.4 M Tris HCl, pH 7.5) for 5 minutes and later rinsed with PBS. The slides were stained with 80 µl of ethidium bromide (final concentration 2 µg/ml) for 5 minutes. The slides were rinsed in ice cold PBS and covered with a cover slips. The prepared slides were observed using fluorescent microscope under green fluorescence light for scoring 100 nuclei. The extent of DNA damage was assessed using a visual

classification method. Cell nuclei were analyzed and given a score from 0 (undamaged nucleus) to 4 (severely damaged nucleus). The slides were prepared in duplicate for each sample and the whole procedure was carried out in dim light to minimized artefactual DNA damage.

Data analysis

Ames test

The numbers of induced and spontaneous revertant colonies in each plate were counted and presented as mean (M) ± standard error of mean (SEM). A nonstatistical method of data analysis was further used to evaluate mutagenicity ratio (MR) by following the formula described by [10]. $MR = \frac{SR+IR}{SR}$ Where SR is spontaneous revertant colonies and IR is the number of induced revertant colonies. The test extract will be judge as positive for mutagen when (a) It produces a dose-dependent increase in the number of *His*⁺ revertant colonies to a level greater than or equal to two-fold ($MR \geq 2$) of the negative control value (b) the dose-dependent increase in revertant colonies was reproducible.

In vivo rodent bone marrow micronucleus and alkaline comet assay

The data generated from these assays were expressed as mean plus or minus standard error (Mean ±SEM). The data were further analyzed for statistical significance using one-way analysis of variance (ANOVA) and the differences between the mean values were compared using Tukey Kramer multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSION

The assessment of balance between the therapeutic and toxicological effects of herbal products is essential with regard to awareness for safety of natural products and due to the high demand by consumers for fresh and natural products as alternative to synthetic additives as important sources of a pharmacological drug. Therefore, determination of the potential mutagenic and genotoxic effects of any useful medicinal plant is necessary for it to be used in therapy. *S. marginatum* was reported to rich in bioactive compounds with numerous therapeutic potentials such as anticancer, anti-inflammatory, antioxidant, antimicrobial and antidiabetic activity in living system [9], but there is no available literature with regard to pre-clinical genotoxicity studies of its crude extract. In view of that, we conducted a battery of genetic toxicology assay viz., Ames test, *in vivo* rodent bone marrow micronucleus and alkaline comet assay to explore the possible mutagenic and genotoxic risk of the algal extract.

Ames test

Bacterial reverse mutation assay was performed in five *His*⁻ mutant tester strains of *S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 using five different concentrations 312.5, 650, 1250, 2500, 5000 µg/ plate of methanolic extract of *S. marginatum* both with and without metabolic activation. The result obtained was presented in (Table 1). Based on the result obtained, no two-fold ($MR \geq 2$) increase in the number of histidine positive (*His*⁺) induced revertant colonies observed in each treatment plate compared to the negative control treatment. The result also revealed that the test extract did not produce a dose-dependent reproducible increase in the number of *His*⁺ induced revertant colonies at the highest dose of 5000 µg/plate in all the five tester strains both with and without metabolic activation. The number of *His*⁺ revertant in positive controls for each strain tested resulted within the expected range.

The bacterial reverse mutation assay is commonly employed as an initial method for screening genotoxic activity of a chemical compound particularly point mutation. The test uses amino-acid requiring tester strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs [3]. Crude extract of *S. marginatum* was used for this assay instead of a single bioactive compound of the test extract which is the compound reported to have potential anticancer activity [9].

Our findings in this study were compared with those that evaluated the mutagenic effect of its functional polysaccharides as compound with suspected risk. Fucoidan is a type of complex sulfated polysaccharide mainly found in the cell wall matrix of various brown seaweeds species with numerous therapeutic applications particularly anticancer and anti-inflammatory activity [15]. In a similar study, reported that no mutagenic activity was detected when low molecular weight fucoidan a sulfated polysaccharide extracted from brown seaweed *Laminaria japonica* was assayed for its mutagenic activity in five *His*⁻ mutant strains of *S. typhimurium* using Ames test [16]. Our result was also in agreement with what reported by [17] that fucoidan extracted from sporophyll of *Undaria pinnatifida* was assayed in *His*⁻ tester strain of *S. typhimurium* and *E. coli* tester strains both in the presence and absence of metabolic activation and was found to be nonmutagenic.

It was known that a large number of chemicals are metabolically transformed *in vivo* and their intermediates in xenobiotic metabolism can themselves be potentially reactive metabolites which can exhibit genotoxic effect [11]. Therefore, we used external metabolic activation system of S9 mixture in the assay in order to increase the chances of detecting promutagens that might likely be present in the test extract. *In vitro* genotoxicity assay is not sufficient enough to confirm the potential genotoxicity of a test substance alone rather, it must be supported with an *in-vivo* test in order to adequately cover factors related to pharmacokinetics and DNA repair processes which were absent in bacterial tester strains and would give accurate genetic end points of the assay [18]. Considering this we supported our *in vitro* mutagenic study with *in vivo* rodent bone marrow micronucleus and comet assay.

In vivo rodent bone marrow micronucleus assay

Methanolic extract of *S. marginatum* was evaluated for its potential clastogenic activity in mice bone marrow erythrocytes using micronucleus assay and the results were presented (Table 2). There exists no significant decrease in PCE and NCE ratio when compared between the vehicle control group and *S. marginatum* treated groups (500, 1000, and 2000 mg/kg BW) indicating that the test extract was not cytotoxic to bone marrow erythrocytes. In addition, no statistically significant increase in the frequency of micronucleus polychromatic erythrocytes (MNPCE) and total micronucleus per cent in *S. marginatum* treated groups were observed over the negative control group at both 24 hrs and 48 hrs sampling interval, confirming that the

test extract was not clastogenic. Single i/p administration of CP (50 mg/kg BW) to positive control group exhibited a significant induction of micronuclei in PCE cells and MN per cent when compared to that of negative control both at 24 hrs and 48 hrs sampling periods.

In a similar study, the potential genotoxic effect of fucoidan obtained from *Undaria pinnatifida* was investigated in bone marrow erythrocytes of Sprague-Dawley rats using *in-vivo* bone marrow micronucleus assay where no significant increase in the frequency of micronucleus PCE was observed and hence reported to be nongenotoxic [19]. Moreover, *in vitro* cytokinesis block micronucleus assay was used to assess the potential genotoxic and cytotoxic effects of brown algae *Dictyopteria membranacea* in human C3A cell lines and it was reported to be nongenotoxic both in the presence and absence of external metabolic activation [20].

In vivo alkaline comet assay

Methanolic extract of *S. marginatum* was evaluated for its potential DNA damaging effects using *in-vivo* alkaline comet assay at 24hrs and 48hrs sampling time. The data obtained were tabulated and a visual scoring system of 0 (normal) to 4 (severe damage) was used in classifying the level of DNA damage (Table 3). There exists no statistically significant DNA damage observed in all the treatment groups with regard to negative control group at both 24 hrs and 48 hrs sampling time. Both negative and treated groups exhibited primarily type 0 and 1 comets form while type 2, 3, and 4 were found to be insignificant.

DNA damage is one of the most important consequences of oxidative stress in the living cells upon exposure to genotoxic agents and when DNA repair system was compromised, it may be unable to modify these inducible damages and genomic instability may lead to mutation, cancer, aging and many other degenerative diseases [21]. Alkaline comet assay is a common and versatile method which provides a rapid visual method for assessing the level of DNA damage quantitatively in a single cell and can be applied to living eukaryotic cells of almost all species using both *in-vitro* and *in-vivo* testing system [14]. The result obtained from this study was compared to what reported by [22], who evaluated the potential DNA damaging effect of a brown seaweed *Fucus vesiculosus* in human lymphocytes using *in-vitro* testing protocols of comet assay and was found not to induce a significant DNA damage in the treated human lymphocytes.

Table 1: Evaluation of mutagenic effect of methanolic extract of *S. marginatum* in *His*⁻ mutant strains of *S. typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) using Ames test

Strains	Test chemicals	Concentration (µg/plate)	Number of revertant (Mean ± SME)/ plate and MR			
			-S9 (Mean±SME)	MR	+S9 (Mean±SME)	MR
TA98	DMSO	-	32.67 ± 1.86	-	30.33 ± 1.45	-
	SM	312.5	34.67 ± 1.45	1.06	36.67 ± 0.88	1.12
	"	625	31.33 ± 1.20	0.96	33.33 ± 0.88	1.10
	"	1250	33.33 ± 0.88	1.02	35.67 ± 0.67	1.18
	"	2500	30.67 ± 1.45	0.94	30.67 ± 2.19	1.01
	"	5000	27.67± 1.20	0.85	29.33 ± 1.20	0.97
	2-NF ^a / B[a]P ^b	1 ^a / 5 ^b	265.00 ± 2.00	-	237.00 ± 1.15	-

TA100	DMSO	-	130.33± 1.20	-	126.33 ±2.03	-
	SM	312.5	134.67± 0.33	1.03	130.67 ±1.67	1.03
	"	625	131.33± 1.86	1.01	133.00 ±1.15	1.05
	"	1250	136.67± 2.19	1.05	129.33 ±1.20	1.02
	"	2500	130.33± 2.40	1.00	130.67 ±1.45	1.03
	"	5000	127.33± 2.60	0.98	125.33 ±0.88	0.99
	NaN ₃ ^a / B[a]P ^b	0.5 ^a / 5 ^b	994.00 ± 2.08		1005.00± 1.73	-
TA102	DMSO	-	238.00 ±2.31	-	243.67 ± 2.40	-
	SM	312.5	241.67 ±2.40	1.02	254.00 ± 0.58	1.04
	"	625	246.33 ±2.33	1.04	259.67 ± 1.76	1.07
	"	1250	243.67 ±2.40	1.02	256.00 ± 1.15	1.05
	"	2500	238.00± 1.53	1.00	260.33 ± 1.76	1.07
	"	5000	233.00± 1.00	0.98	261.00 ± 1.15	1.07
	MMC ^a / B[a]P ^b	0.5 ^a / 5 ^b	1110.00 ± 5.03	-	1269.00 ± 0.58	-
TA1535	DMSO	-	22.67 ± 2.60	-	22.00 ± 0.58	-
	SM	312.5	19.00 ± 2.08	0.84	21.00 ± 1.15	0.95
	"	625	24.20 ± 1.15	1.06	22.00 ± 1.15	1.00
	"	1250	25.00 ± 1.73	1.10	24.67 ± 1.20	1.12
	"	2500	25.33 ± 1.20	1.12	24.33 ± 0.88	1.11
	"	5000	24.67 ± 0.88	1.09	26.00 ± 0.58	1.18
	NaN ₃ ^a / B[a]P ^b	0.5 ^a / 5 ^b	320.00 ± 2.65	-	294.00 ± 2.08	-
TA1537	DMSO	-	11.00 ± 0.58	-	13.00 ± 1.15	-
	SM	312.5	12.00 ± 1.00	1.09	15.33 ± 0.88	1.18
	"	625	14.00 ± 0.58	1.27	14.67 ± 0.33	1.13
	"	1250	13.33 ± 1.86	1.21	15.00 ± 1.53	1.15
	"	2500	14.67 ± 0.88	1.33	13.33 ± 1.20	1.03
	"	5000	15.00 ± 0.58	1.36	12.00 ± 1.15	0.92
	9-AA ^a / B[a]P ^b	50 ^a / 5 ^b	143.00 ± 1.73	-	113.00 ± 1.15	-

DMSO: vehicle control, SM: *Stoechospermum marginatum* extract, SME: standard error of mean, S9: soluble liver microsomal enzymes fraction, MR: mutagenicity ratio, NaN₃ sodium azide, 2-NF: 2-nitrofluorine, MMC: mitomycin C, 9-AA: 9-aminoacridine, Benzo[a]pyrene B[a]P, a: in the absence of -S9, b: in the presence of +S9.

Table 2: Evaluation of clastogenic effect of methanolic extract of *S. marginatum* in mice bone marrow erythrocytes using *in vivo* micronucleus assay

Treatments	Sampling Time (hrs.)	Dosage (mg/kg/Day)	Total MNPCE Mean ±SEM	MNPCE/2000 PCE Mean ±SEM (%)	PCE/PCE+NCE Mean ±SEM (%)
NC	24hrs	-	1.60±0.40	0.08±0.02	74.50 ±0.57
G1	24hrs	500	2.20 ±0.66	0.11 ±0.03	73.20 ±0.68
G2	24hrs	1000	2.40 ±0.68	0.12 ±0.03	70.10 ±0.51
G3	24hrs	2000	3.00±0.45	0.15 ±0.02	68.30 ±0.47
NC	48hrs	-	1.40 ±0.24	0.07 ±0.01	75.80 ±0.51
G1	48hrs	500	1.80±0.58	0.09 ±0.03	74.60 ±0.80
G2	48hrs	1000	2.60±0.51	0.13 ±0.03	71.80 ±0.72
G3	48hrs	2000	2.80±0.66	0.14 ±0.03	70.00 ±0.50

NC: vehicle control, G: group, NCE: normochromatic erythrocytes, PCE: polychromatic erythrocytes, MN: micronucleus, SEM standard error of mean. P<0.05, n = 5

Table 3: Evaluation of DNA damaging effect of methanolic extract of *S. marginatum* using *in vivo* alkaline comet assay

Treatments	Dosage (mg/kg)	Time	DNA scores mean \pm SEM					Total DNA damage Mean \pm SME
			0	1	2	3	4	
NC	-	24hrs	94.20 \pm 0.86	3.40 \pm 0.93	2.40 \pm 0.24	0.00 \pm 0.00	0.00 \pm 0.00	5.80 \pm 0.86
G1	500	24hrs	89.40 \pm 1.08	6.60 \pm 0.51	2.60 \pm 0.68	0.80 \pm 0.37	0.60 \pm 0.40	10.60 \pm 1.08
G2	1000	24hrs	88.00 \pm 1.18	7.00 \pm 1.14	3.20 \pm 0.37	1.40 \pm 0.68	0.40 \pm 0.24	12.00 \pm 1.18
G3	2000	24hrs	86.60 \pm 1.03	7.60 \pm 0.75	3.00 \pm 0.32	1.80 \pm 0.37	1.00 \pm 0.32	13.40 \pm 1.03
NC	-	48hrs	92.60 \pm 1.50	4.40 \pm 1.29	3.00 \pm 0.32	0.00 \pm 0.00	0.00 \pm 0.00	7.40 \pm 1.50
G1	500	48hrs	91.00 \pm 0.71	6.40 \pm 0.81	1.60 \pm 0.60	0.80 \pm 0.37	0.20 \pm 0.20	9.00 \pm 0.71
G2	1000	48hrs	89.00 \pm 0.71	5.80 \pm 0.37	3.40 \pm 0.51	1.00 \pm 0.32	0.80 \pm 0.37	11.00 \pm 1.18
G3	2000	48hrs	87.80 \pm 1.14	7.80 \pm 0.80	2.60 \pm 0.24	2.00 \pm 0.32	0.60 \pm 0.24	13.20 \pm 1.14

NC: negative control, G: group. $P < 0.05$, $n = 5$

CONCLUSION

This study is a novel work that assessed the potential mutagenic and genotoxic effects of methanolic extract of *S. marginatum* using *in-vitro* mutagenicity and *in-vivo* genotoxicity assay. The results obtained clearly confirmed that the test extract was devoid of causing any mutagenic or genotoxic damage to living cells genome under the condition tested. Therefore, the test extract is safe and can be utilized by pharmaceutical, nutraceutical and chemical industries for its relevance applications.

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Conflict of interest

The authors have read and approved the final draft of this manuscript and have no conflict of interest to declare with regards to publication of this manuscript.

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