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Effectiveness and Efficiency of Monosodium Glutamate as a Potential Mutagen Inducing Polyploidy in Urginea Indica Kunth

RICHA SINHA (richa.sinhaa05@gmail.com)
AMITY University Jharkhand https://orcid.org/0000-0003-2910-1828

Research Article

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Posted Date: February 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-188729/v1

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Effectiveness and efficiency of Monosodium Glutamate as a potential mutagen inducing polyploidy in *Urginea indica* Kunth

Dr. Richa Sinha
AMITY University Jharkhand
rsinha1@rnc.amity.edu

Abstract

Monosodium Glutamate is a food additive well-known as a flavor enhancer being used extensively in food industries. Also, it has long been used as a cheap source of fertilizer owing to the presence of high nitrogen contents in it. Furthermore, it also shows genotoxic effects on plants. Hence, in the present study the genotoxic properties of Monosodium Glutamate had been explored to assess its potential as a mutagen. The objective was specifically to find out its potential in inducing polyploidy in plants. For this, a medicinal plant, *Urginea indica* Kunth with immensely high therapeutic value was selected. The effectiveness and efficiency of Monosodium Glutamate on *Urginea indica* Kunth was assessed based on the records obtained after treatments in two generations, viz. M_1 and M_2 Generations. Its effectiveness and efficiency on *Urginea indica* Kunth pave a way for its use as a potential mutagen which can induce genetic variability among the selected wild species of the plants with great ease. Moreover, the higher degree of polyploidy obtained in the M_2 generation established the objective of the experiment, that Monosodium Glutamate could be used with controlled, adequate dose and maintained experiment setup in inducing polyploidy in plants.

Key words: Monosodium Glutamate (MSG), *Urginea indica* Kunth, polyploidy, chemical mutagen, Mutagenic effectiveness and efficiency

Introduction

The gigantic collection of vegetation embraces a promising medicinal plant, *Urginea indica* Kunth. The worthiness of this plant was recognized ever since its discovery. Commonly it is called as wild onion, the name which had been derived from a Sanskrit word '*Palandu'* as defined in Garura Puran [132]. The preliminary record of using *Urginea indica* Kunth as a medical plant was mentioned in Egyptian Ebers Papyrus (1500 BC) [130]. Since then, even in the modern pharmacology, it had been used extensively to cure innumerable human ailments. It belongs to the Liliaceae family [33, 132] having commercially high therapeutic status involving its use in human homeopathy, phytotherapy and even in veterinary science. Moreover, it also possesses significantly high levels of nutrients [129].

Although entire plant part is of therapeutic values, the bulbs of *Urginea indica* Kunth are of vital significance owing to the presence of bioactive compounds with a potential to cure numerous human disorders, viz. cancer, respiratory trouble, bronchitis, stomach ache, paralysis, rheumatism, leprosy, skin diseases, infectious wound, whooping cough, arthritis, tumours, edema, male sterility, gout, chronic cough, psoriasis, swellings, pulmonary troubles, expectorant, diuretic properties, and cardiac tonic etc. [59, 111, 127, 34, 1, 114, 21]. Besides, they also possess the antifungal properties with varied contents of phytochemicals [108]. Nevertheless, *Urginea indica* Kunth are wild herb prevalent mostly in remote areas being sparsely accessible. They are endemic to certain floristic regions of India, Africa and Mediterranean regions [6]. Moreover, therapeutic potential had led to their overexploitation both as a source of traditional medicine as well as a potential raw material by pharmaceutical companies making the plant vulnerable in the wild [91, 70]. Consequently, immensely high therapeutic values and vulnerable status of *Urginea indica* Kunth makes the induction of genetic variabilities and hybrid production vital for it through mutation breeding. Wherein, the genetic variabilities would lead to increased yield of the plant while hybridization would produce polyploidy. Both the changes thus produced would enable the extraction of the principle medicinal prospective from *Urginea indica* Kunth.

Mutation breeding leads to a significant change in the specific traits of the target plants allowing their other phenotypic traits to remain intact. It enables the introduction of multiple traits to induce genetic variabilities among the species for the preparation of future database. [82]. This makes mutation breeding a dynamic and at the same time efficacious tool for the improvement of plant traits [3] aiding their conservation through enhancement of prevailing germplasms [36]. Furthermore, it is a means of studying the characteristics and functions of specific genes in the plants which can be used for advancement of the traits in economically important plants [5], thereby significantly increasing their production rate [74].

Mutation breeding involves the use of chemical as well as physical mutagens for the induction of genetic variabilities among the species. Specific mutagens are required for obtaining the desired traits by altering the genes causing spontaneous mutation. This in response can help in establishing permanent heritable changes in the offspring, which can be exploited by the plant breeders as a genetic variability for further processing [105]. Chemical mutagens are preferable in plant breeding owing to their ability of causing point mutations. The ease with which they can be used in the induction of genetic variability among the species without damaging their other traits, make them ideally suited for any plant breeding programs. The chromosomal aberrations caused by the chemical mutagens after treatment, are the reliable source of estimating the effects of chemical mutagens on the plants. Any cytotoxic and genotoxic effects caused by the mutagens involved in the experiment signifies the induction of mutation in the plants. Besides, efficiency and effectiveness of the mutagens is of utmost importance in the fruitful mutation breeding. This allows the higher occurrence of mutations, resulting in the production of desired traits of the plants [113, 140, 90]. The potential of any mutagens thus, can be determined through the assessment of their effectiveness and efficiency.

If we look back at the history of mutation breeding, the very first chemical to be used as a mutagen was mustard gas for the treatment of *Drosophila melanogaster* in the year 1940 [15, 16]. Since then several chemicals had been discovered with mutagenic potential but only a few had established their position in effective use. Consequently, in the recent trends, the chemical mutagens being effectively used in any mutation breeding programs, involves the selection of only a limited number of mutagens. Keeping this in view, present research involved the use of a food additive, Monosodium Glutamate, to determine its efficiency as a potential mutagen. The objective was thus to determine the sensitivity of the plant to different concentration treatments of Monosodium Glutamate.

Monosodium Glutamate is a sodium salt which is a derivative of the amino acid, Glutamate, found naturally in almost all the food that we consume. It is being used as a flavor enhancer especially in Asian cuisines [71, 104]. It is a crystalline, white, odorless salt like powder which is easily soluble in water. A Japanese biochemist, Kikunae Ikeda was the first to accidently prepare the Monosodium Glutamate in 1908, while isolating the savory taste of the edible seaweeds [86]. Although it is found abundantly in the fermented products of proteins, commercially it is being produced by the molasses through fermentation processes. The discovery of Monosodium Glutamate led to its widespread traditional use as a flavor enhancer which provides the fifth flavor '*Umami'* increasing the savory taste of the food products manifold [23]. Moreover, it is metabolized by human body exactly in similar manner as the Glutamate because of the resemblance of the two [22].

Although safety of Monosodium Glutamate on human health have already been established and discussed in the plethora of research articles, it still is a matter of debate among different group of scientists. For some group of researchers, it has long been accompanied with toxicity including its genotoxic effects on plants while several metabolic disorders in human body ranging from obesity to Chinese Restaurant Syndrome and several neurotoxic effects [103]. Its genotoxic effects had already been shown on *Allium cepa* [62]. However, other group of researchers found it to be entirely safe for human consumption. Many researchers have failed to proof the toxic effects of Monosodium Glutamate on human body. Furthermore, FDA (Food and Drug Administration) had interpreted its safety based on the FASEB report [117]. Although Food safety regulatory agencies' consideration of Monosodium Glutamate safety is based on the massive experiments, further controlled and well planned clinical and epidemiological studies for its safety is needed [152].

Nevertheless, it had already established its worthiness as a fertilizer in agricultural field through the massive trials. The powdered commercial form of Monosodium glutamate is considered as a cheap, odourless fertilizer being extensively used in agricultural field for fast growth rate of plants because of its high nitrogen contents [78]. Moreover, it is also reported to have the potential to help in the growth of beneficial soil microbes [51]. The Monosodium Glutamate had also been used successfully as a powdered fertilizer for growing corn plants efficiently in the field. Thus, it could also be used as an alternative and sustainable fertilizer [122]. Additionally, the use of Monosodium glutamate industrial wastewater, as a fertilizer providing nitrogen and other organic matter to expedite the plants growth in the field had also been recognized [138].

Apart from that, Monosodium Glutamate also finds its place in inducing high intensity genotoxic and cytotoxic effects on plants. The genotoxic effects of Monosodium Glutamate have already been demonstrated in many plants [62, 116, 80, 146, 99].

Considering above-mentioned facets, the present research work was carried out to assess the other potentials of Monosodium Glutamate as a mutagenic agent by studying its mutagenic effectiveness and efficiency on *Urginea indica* Kunth.

Material and Methods

Sample collection

There are different varieties of *Urginea indica* Kunth prevalent in Ranchi, Jharkhand. The different varieties show great morphological variations with mostly similar chemical composition and constant chromosome numbers. The peculiar traits which differentiates these plants into two major types is based on their flowering style. The first group of plants shows the growth of flowers and shoot simultaneously while in the second group, at first shoot emerges which is followed by the advent of flowers; and the two are named as Hysteranthus and Synanthus varieties respectively [131]. In this research work, the bulbs of Hysteranthus variety of *Urginea indica* Kunth (Fig. 1) were collected from Birsa Agricultural University (BAU), and the healthy bulbs of uniform sizes were selected for the experiment.

Experimental layout

The experimental observations were based on the root tip systems of *Urginea indica* Kunth and the experiment was performed in two parts, wherein data were prepared for two generations, M_1 *and* M_2 in similar manner. Consequently, the plants after treatment with Monosodium Glutamate were raised for the two generations. The definite experimental plots were prepared for raising the two generations. The observations after slide preparation were recorded for M_1 generation. Afterwards, the treated bulbs of M_1 generation were planted in the new experimental plots and allowed to complete one flowering season, i.e. one year. Eventually, the bulbs were taken out and prepared for raising M_2 generations by removing the roots, then washing the bulbs thoroughly with running water and planting them back in the new experimental plots. Rooting of the bulbs were allowed and thereafter slides were prepared to record the data for M_2 generation.

Bulbs treatment and mitotic preparation

Uniform sized fresh bulbs of *Urginea indica* Kunth (Fig. 2) were selected and their roots were removed completely with scalpel. Five different concentrations of Monosodium Glutamate were prepared, viz. 0.1g/l, 0.2g/l, 0.3g/l, 0.4g/l and 0.5g/l. The bulbs were then soaked discretely in different concentrations of Monosodium Glutamate for six hours, subsequently the bulbs were washed carefully with running tap water for few minutes. Finally, the treated bulbs were planted in the prepared plots to raise M_1 Generation. After few days fresh root tips (~1-2cm long) of the bulbs were obtained from the bulbs of specific concentrations, and prepared for the observation in following chronological steps:

i. **Pretreatment:** The excised roots were pretreated with 1,4 Paradichlorobenzene for four hours
ii. **Fixation:** The roots were then fixed in the freshly prepared fixative (1:3 Glacial Acetic-Alcohol- Solution with a pinch of Ferric chloride) for 24 hours
iii. **Preservation**: Fixed roots were then transferred in 70% Alcohol (Ethanol)
iv. **Hydrolysis**: 1 N HCL for five to ten minutes based on thickness of roots
v. **Staining**: Root tips were warmed in 2 per cent Acetocarmine for few minutes
vi. **Slide preparation**: Slides were prepared after staining, by Squash technique.

Data analysis

The aberrations in the root tip cells of both M_1 and M_2 generations were recorded under microscope using the magnification of 45X. Approximately 500-1000 cells were observed for each concentrations of Monosodium Glutamate to analyse data and prepare the tables. Photographs of *Urginea indica* Kunth bulbs and photomicrographs of the prominent chromosomal aberrations were taken from digital SLR Nikon camera.

The cytotoxicity (CT), genotoxicity (GT), mutagenic effectiveness and efficiency of all the five concentrations of Monosodium Glutamate in *Urginea indica* Kunth were assessed for both the generations (M_1 and M_2). Mutagenic Rate (MR) for both the generations were also included for the assessment.

Cytotoxicity and genotoxicity

Cytotoxicity (CT) and genotoxicity (GT) assessment was done based on the methods of Asita et al. (2017) [14]. The cytotoxicity was measured by mitotic index (MI), wherein mitotic index is expressed in percentage as:

$$\text{Mitotic Index (MI)} = \frac{\text{Total Number of dividing cells}}{\text{Total number of cells observed in the field}} \times 100$$

Genotoxicity (GT) was measured as the number of aberrant mitotic cells (AMC) per 100 mitotic cells [viz. AMC + normal mitotic cells (NMC)].

$$\text{Frequency of GT} = \frac{\text{AMC}}{(\text{AMC} + \text{NMC})} \times 100$$

Where,

CT = Cytotoxicity
MI = Mitotic Index
GT = Genotoxicity
AMC = Aberrant Mitotic Cells
NMC = Normal Mitotic Cells

Effectiveness and efficiency

The effectiveness and efficiency of each concentrations of Monosodium Glutamate on *Urginea indica* Kunth were measured based on the formulae given by Konzak et al., (1965) [77].

$$\text{Mutagenic Effectiveness} = \frac{\text{Percent Mutagenic Frequency}}{\text{Mutagen Dose or (Concentration} \times \text{Time)}}$$

$$\text{Mutagenic Efficiency} = \frac{\text{Percent Mutagenic Frequency}}{\text{Biological Damage}}$$

Where,

- ✓ Mutagenic dose of a chemical mutagen is calculated as a product of concentration and treatment [Concentration (c) × treatment duration (t)]
- ✓ Biological damage caused by Monosodium Glutamate in *Urginea indica* Kunth was considered based on Mitotic Inhibition (MI in control/MI in treated cell×100)

$$\text{Mutation Rate} = \frac{\text{Sum of values of efficiency or effectiveness of particular Mutagen}}{\text{Number of treatment of particular Mutagen}}$$

Where,

MI = Mitotic Index

Mutation rate gives the insight of mutation induced by specific mutagen (here, Monosodium Glutamate) independent of dose or concentrations involved. It explains the retention of the induced mutation in M_2 generation also. Thus, potential of the mutation involved can be assessed based on mutation rate.

RESULT:

The effects of Monosodium Glutamate (MSG) treatment on *Urginea indica* Kunth were observed and assessed for the two generations, M_1 and M_2. The major findings with statistical analysis are depicted in Table I-III, Fig, 1-14.

Chromosomal aberrations, cytotoxicity, genotoxicity, mutagenic effectiveness and efficiency of Monosodium Glutamate (MSG) at all the concentrations were assessed for both the generations, i.e. M_1 and M_2, of *Urginea indica* Kunth. Mutation Rate independent of the concentrations of Monosodium Glutamate were also calculated.

Chromosomal aberrations

Several types of chromosomal aberrations (Fig. 3-14) were induced by Monosodium Glutamate at different stages of mitotic divisions in the root tip cells of *Urginea indica* Kunth in M_1 as well as M_2 generations. Mostly similar chromosomal aberrations were observed in both the generations with varied frequencies at each treatment. All the observed chromosomal aberrations exhibited dose dependent increase in M_1 generation, which declined

considerably in the M_2 generation (Table I and II). In M_1 generation, maximum aberrations were induced by Monosodium Glutamate at metaphase stage followed by telophase and subsequently at anaphase stage. In contrast, no chromosomal aberrations were observed at prophase stage.

The common chromosomal aberrations observed at each mitotic stage can be summarized as follows:

i. *Prophase:* No aberrations observed

ii. *Metaphase:*

Precocious movement of chromosomes (Fig. 10)**,** sticky metaphase (Fig. 11, 12)**,** ball metaphase, tropokinesis (Fig. 5)**,** nucleolus at metaphase**,** diagonal metaphase**,** polyploidy (Fig. 3-4)**,** chromosome fragments**,** translocation ring (Fig. 9).

iii. Anaphase:

Disturbed anaphase (Fig. 13)*,* laggards*,* bridges**,** multipolar anaphase*,* unequal separations (Fig. 14).

iv. Telophase

Nuclear lesions, binucleate (Fig. 7), multinucleate cells (Fig. 8), micronuclei (Fig. 11), telophase bridges, elongated cells (Fig. 6).

In M_1 generation, highest frequencies of chromosomal aberrations were observed at metaphase and telophase stages. Elongated cells, chromosomal fragments, sticky metaphase nuclear lesions and multinucleate cells were the most abundant aberrations observed at different concentrations of Monosodium Glutamate (table 1). The number of aberrant cells increased linearly at higher concentrations of Monosodium Glutamate (MSG).

In M_2 generation, the frequency of chromosomal aberrations declined significantly at each treatments of Monosodium Glutamate. *However, the frequency of polyploidy in M_2 generation was observed to increase significantly at all the concentrations, although the presence of Polyploids in M_1 generation were comparatively very low.* Similarly, the frequency of elongated cells also increased significantly in M_2 generation (Table I and II).

Cytotoxicity and Genotoxicity

Cytotoxicity of Monosodium Glutamate on *Urginea indica* Kunth were assessed based on Mitotic index which declined linearly in a dose dependent manner in M_1 generation, with a slight increase in the M_2 generation (Table III).

Genotoxicity in M_1 generation was noticed to increase significantly in linear fashion with the higher concentrations. However, the genotoxicity in M_2 generation was observed to be relatively lower as compared to that of M_1 generation (Table III).

Mutagenic effectiveness, efficiency and mutation rate

Mutagenic effectiveness in both M_1 and M_2 generation was reported to decrease with higher concentrations. However, the effectiveness in M_2 generations was slightly lesser than that of M_1 generation at all the concentrations.

In M_1 generation, varied degree of mutagenic efficiency was observed at different concentrations of Monosodium Glutamate. The increase or decrease in the efficiency was reported not to be concentration dependent. It was reported to be highest at the concentration 0.1g/l while lowest at 0.4g/l concentration treatment (Table III). Similarly, in M_2 generation the mutagenic efficiency was observed to be varying at different concentrations, but it was comparatively higher as compared to M_1 generations at all the concentrations except at 0.3g/l treatment. Mutagenic efficiency at 0.1g/l concentration, was significantly higher in M_2 generation (4.256) as compared to M_1 generation (2.878) [table III].

Mutagenic rate was calculated based on effectiveness as well as efficiency, for both the generations. In M_1 generation, mutation rate based on effectiveness was slightly higher as compared to that at M_2 generation, while the mutation rate based on efficiency was comparatively lower in M_1 generation as compared to M_2 generation (table III).

Discussion

Chromosomal aberrations

The potential of Monosodium Glutamate as a mutagen was assessed through its genotoxicity and cytotoxicity on *Urginea indica* Kunth, for which the root tip cells were used in the experiment. Choice of such cells in plants is considered ideal for the quick and subtle chromosomal assessments after mutagenic treatments. Chromosomal aberration rates induced in the root tip cells (somatic cells) is considered reliable and standard means for the assessment of sensitivity of plants to the selected mutagen [63]. Such chromosomal aberrations by mutagens are associated with the evidence of genetic toxicity induced by the mutagens [73] and hence, are regarded as the base

for genetic changes produced in the plant assays. They are considered as the principle indicator of potential changes induced by the mutagens which can determine the clastogenic effects of the mutagens thereby permitting the exchange of chromosomes with successive genetic damages [150]. Moreover, the appropriate and effective dose treatments of the mutagens can be assessed through chromosomal aberrations to ensure the maximum outcomes from the breeding program [97]. Hence, in the present research work the genotoxic and cytotoxic effects of Monosodium Glutamate on *Urginea indica* Kunth were confirmed through the induction of significantly higher frequency of chromosomal aberrations.

For confirmation of the mutation induced by Monosodium Glutamate in the *Urginea indica* Kunth, the frequencies of chromosomal aberrations were evaluated for two generations i.e. M_1 and M_2 generations. Significantly higher frequencies of aberrations were recorded in M_1 generation which increased in linear fashion with increased concentrations. These increased frequencies were reported to be the result of increased ploidy level which in return led to the increased accessibility in the marked zones for mutation. Such increased frequencies of aberrations induced by the chemicals ensure the improvement in the traits of the plants being used. Although all the chromosomal aberrations are not beneficial for the plants, the beneficial effects of the mutagens can be examined and utilized by the researchers [60].

Some of the chromosomal aberrations were reported to decline in M_2 generation showing that the induced mutation can be the type of damage which could easily be repaired and prevented from passing to the next generation. However, some of the aberrations are not subjected to any repair mechanism which may pass to the next generation for storage and expression as spontaneous mutation in plant offspring [105]. This aligns with the present finding that some of the aberrations increased significantly in M_2 generation, which was comparatively lower or negligible in the M_1 generation. Moreover, such increase may also be the result of the fact that many of the induced mutations remain recessive in M_1 generation but can be expressed in the next generation [13, 106].

In M_1 generation, maximum frequencies of chromosomal aberrations were noticed at metaphase stage with chromosome fragments and sticky metaphase being the most prominent one. The presence of chromosomal fragments was reported to be the result of chromatids and chromosomes stretching at metaphase and breakage at those very sites [29]. Formation of such fragments are the indicator of fatal clastogenic effects caused by the mutagens [151]. Anaphase or telophase bridges may also end up with the formation of chromosome fragments [124, 137] or the stickiness at metaphase can also be the vital cause of fragments formation [2]. Similarly, the aberration, sticky metaphase (Fig.12) induced by Monosodium Glutamate is generally considered as an irreversible damage killing the cell [47, 42, 46, 146]. Stickiness of the chromosomes led to the improper folding of chromatin by preventing the cell cycle to proceed further and get arrested at metaphase stage [41]. The stickiness at metaphase can be caused by several mechanisms initiated by the mutagenic treatment, but one of the vital causes can be the hetero chromatization of a chromosome which denatures nucleic acid, thus making chromosomes to become adhesive [56]. Stickiness can be defined as the physical adhesion of proteinaceous matrix of chromatin material which makes the chromosome to appear as sticky structure, losing its individual entity [109, 20].

Apart from the above-mentioned chromosomal aberrations, many others with slightly less frequencies were also recorded at different concentration treatments. Precocious movement of chromosomes observed (Fig. 10, 12), may be the consequence of univalent chromosome formation at the end of prophase [107]. Monosodium Glutamate might had led to Tropokinesis at metaphase (Fig.5) by producing the abnormal orientation of the spindle fibers or by the impairment of microtubular system [35]. Yet another aberration, diagonal metaphase might be the result of Monosodium Glutamate action in inhibiting ATPase causing spindle inactivation, thus collapsing the spindle apparatus [32]. The formation of the translocation rings by the action of Monosodium Glutamate, were the result of telomeric losses which can be lethal to the cells. These translocation rings were the probable cause of anaphase bridge formation [58].

In M_1 generation, relatively lesser frequencies of polyploids were observed at all the concentration treatments of Monosodium Glutamate which could be attributed to the fact that the induced mutation fails to be expressed in the first generation, as the plants faces maximum physiological disorders caused by the mutagenic treatments. Also, most of the induced mutations here remains recessive. This limits the identification of any genotypic and phenotypic traits in the first generation. However, in M_2 generation, drastic increase in the frequency of polyploids were reported to be the consequence of expression of the recessive traits after restoration of the damages caused in M_1 generation [96]. Production of polyploids could be the result of doubling of the chromosomes in somatic cells induced because of the mitotic inhibition capacity of the Monosodium Glutamate treatment in *Urginea indica* Kunth [92]. Moreover, the spindle disruption along with the microtubule polymerization forming new structures were responsible for the production of polyploids [27].

Many chromosomal aberrations were also observed at anaphase stages after Monosodium Glutamate treatments in both the generations. Disturbed anaphase (Fig. 13) were also observed frequently at all the concentration treatments which might had been caused by the chromosomal irregularities at various mitotic stages

[10, 95]. Disturbance might also be attributed to the complete inhibition of spindle apparatus because of decline in the activity of microtubules present in the spindle fibres [43]. Formation of laggards at anaphase can again be the effect of spindle failure to organize and function normally [109]. There are many other reasons for the formation of lagging strands at anaphase. One of them can be kinetochore failure for attachment with spindle fibres [9]. The formation of chromosomal fragments might be responsible for the lagging strand formations [48]. Appearance of anaphase bridges were reported to be the originator of aneuploids and polyploids after treatment with Monosodium Glutamate [54]. Constant breakage and fusion of chromosomes were responsible for the formation of anaphase bridges [55]. The formation of anaphase bridges can be the symbol of extremely high clastogenic effects of Monosodium Glutamate [57] which can be useful for obtaining information on clastogenic activity. Another aberration observed at anaphase stage was multipolar anaphase, frequently present at all the concentrations, which might had been the result of inhibition of spindle fiber formation [53].

Chromosomal aberrations at telophase were equally very high. Elongated cells, nuclear lesions and multinucleate cells were the marked abnormalities observed in *Urginea indica* Kunth. However, many other aberrations were also observed at telophase with varying frequencies.

Nuclear lesions were observed at a very high frequency, which could be the result of fragmentation of nuclear material by Monosodium Glutamate which had led to subsequent disruption [116]. Binucleate and multinucleate cells (Fig. 7, 8) were also observed abundantly at telophase, which might had resulted from the inhibition of cytokinesis or cell plate formation [26] leading to polyploidy [28]. Formation of multinucleate cells (Fig. 8) was the consequence of RNA disruption and dispersion forming nucleus [100, 133].

The micronuclei (Fig. 11) observed in the present study might had been the result of failure of the fragmented chromosomes to be incorporated into any of the daughter nuclei at telophase, thus, leading to cellular death due to the deletion of primary genes [8]. Presence of micronuclei may direct aneuploidy tailed with cell death [61]. Appearance of micronuclei after treatment, are considered as the indicator of mutagenic potential of the mutagen used [87, 8, 79].

Elongated cells (Fig. 6) with small nuclei reported at all the concentrations might be due to the activity of Monosodium Glutamate interfering with the production rate of DNA, RNA and protein at different stages of cell cycle.

Cytotoxicity and Genotoxicity

Cytotoxicity of a mutagen is measured through Mitotic index, which was reported to decrease with increased concentrations of Monosodium Glutamate in both M_1 and M_2 generations. The mitotic index at each concentration was reported to be comparatively lower as compared to that in control. The decreasing mitotic index after treatment with mutagens are considered as an authentic and dependable criterion for the determination of cytotoxicity [125].

The reduction of Mitotic index results from the inhibition of DNA or protein synthesis or from blockage in G2 phase of the cell cycle [147, 4, 12, 75, 110]. Moreover, reduction in the mitotic index suggested the mitodepressive action of the Monosodium Glutamate, which could have developed by the suppressed DNA or the protein synthesis, preventing the cells from entering mitosis phases [31]. Another probable reason for the lowering of mitotic index might be the significant genotoxicity observed at higher concentrations [116]. Thus, the reduction in mitotic index in *Urginea indica* Kunth with increased concentrations showed the ability of Monosodium Glutamate to inhibit DNA synthesis. Such inhibition of DNA might had been caused by the decreased levels of ATP and pressure [45, 68].

The frequency of chromosomal aberrations indicates the genotoxicity of the mutagens applied [101]. In the present study the genotoxicity was reported to increase with the concentrations in M_1 generations, however, its significant decline in the M_2 generations was the result of repair mechanism undergone by the DNA. The higher degree of genotoxicity thus, confirms the genotoxic effects of Monosodium Glutamate on *Urginea indica* Kunth.

Mutagenic effectiveness, efficiency and mutation rate

Assessment of effectiveness and efficiency of mutagens is considered as an important tool for the successful mutation breeding program. The detailed information of the duo enables the easy selection of the mutagens, assisting the recovery of high frequencies of mutations [139, 81]. Such selection is possible in the initial generations (viz. M_2 and M_3) after treatments. The selected effective and efficient mutagens then can induce massive variability in the desired crops/plants [128]. Genetic variabilities induced by the mutagens can lead to crop improvement which can be used directly for commercial cultivation or can be stored with new genetic traits [118].

The effectiveness and efficiency together determine the usefulness of a mutagen in plant breeding [135]; however, both are completely different. The effectiveness of mutagens never decides its efficiency and vice versa. Thus, effectiveness of mutagens is not an assurance for its being efficient [49, 135].

Mutagenic effectiveness can be defined as the degree of mutations induced by the mutagen per unit dose. It is considered as an indicator of a genotypic response with increased mutagenic doses. Although effectiveness of a mutagen is hypothetically important, it lacks immediate practical inferences. In contrast the mutagenic efficiency is the evaluation of mutation frequencies along with the degree of damages caused by the mutagen in the form of abnormalities and lethality [128, 77].

It is the indicator of comparatively less damage caused by the induced mutation. Higher efficiency of the mutagens ensures comparatively lesser amount of damage caused by the induced mutations [149].

In the present research work, mutagenic effectiveness in M_1 generation, decreased drastically with the increased concentrations of Monosodium Glutamate. It was reported to be comparatively higher at the lower concentration treatments, which exhibited the independence of mutations to the increased concentrations of the mutagens. Meaning the chances of mutations is not relational to the higher concentrations of mutagens [84]. Moreover, the decline in the effectiveness in the M_2 Generation could be the result of elimination of severely damaged cells after M_1 generation. Toxicity of the mutagen in M_1 generation inducing higher frequencies of aberrations might be responsible for the decline of effectiveness. Every concentrations of the Monosodium Glutamate could have its own effectiveness independent to the concentration treatments [136].

In M_1 generation, mutagenic efficiency was reported to be higher at lower concentration treatments showing the lesser toxic effects of the Monosodium Glutamate to the plants [128]. On the other hand, it was reported to increase slightly in M_2 generation independent of the concentrations. Since higher mutagenic efficiency is responsible for lower damages caused to the plants, it therefore, confirmed the restoration of the damages caused in M_1 generation which in return led to the increased efficiency in M_2 generation. However, the mutagenic efficiency in both the generations was comparatively higher in the lower concentrations which could be attributed to the fact that the damages caused with increased concentration are higher than the rate of induced mutation. Moreover, the higher efficiency depends both upon the biological system as well as damages caused at all the levels of the plants, viz. chromosomal aberrations, pollen sterility etc. Lower concentrations of the mutagens with comparatively lesser frequencies of damages might thus, ensure the higher potential of inducing mutations. The selection of efficient mutagens with appropriate concentrations are crucial for any successful breeding programme to ensure lucrative use of the mutagens aimed at the induction of mutations [77]. Even though lesser in volume, but the mutagenic efficiency was observed to increase in M_2 Generation as compared to M_1 generation, which exhibited the efficiency of Monosodium Glutamate to restore the damages and produce the desirable changes in the selected plant, *Urginea indica* Kunth.

Similar findings with higher effectiveness and efficiency had also been reported by many researchers in their experiments, which demonstrated that the lower concentrations of the mutagens are more efficient with lesser damages in comparison to those at higher concentrations [25, 98, 102, 112, 115, 126, 134].

Mutagenic rate was calculated based on effectiveness as well as efficiency, for both the generations. In M_1 generation, mutation rate based on effectiveness was slightly higher as compared to that at M_2 generation, while the mutation rate based on efficiency was comparatively lower in M_1 generation as compared to M_2 generation (table III).

Higher mutation rate with least damages induced by the mutagens in mutation breeding is desirable. However, the higher concentrations of the mutagens inducing highest mutations equally induce higher frequencies of damages [25]. Mutation rate is not dependent upon the specific concentration of the mutagens involved and gives the idea of the mutation induced [94]. The mutagenic effectiveness and efficiency of mutagens are likely to alter the mutation rate depending upon the various external factors [76]. The increased mutation rate in M_2 generation in terms of efficiency showed the mutagenic potential of Monosodium Glutamate.

The above findings align with the objective of the research which was carried out to assess the effectiveness and efficiency of the Monosodium glutamate on *Urginea indica* Kunth. The bulbs of *Urginea indica* Kunth is a rich source of secondary metabolites making it a plant of immensely high therapeutic value. Root cells obtained from their bulbs after treatment with Monosodium Glutamate were subjected to the investigation for first mitotic cycle which is considered a quick means of determining mutagenic effects. Such investigation are well established methods of providing supplementary information about a new mutagen used in a mutation breeding programme through chromosomal studies [96]. The results obtained exhibited the potential of Monosodium glutamate as a mutagen, where higher concentration treatments were toxic causing lethal or irreversible damages to the chromosomes, while the lower or the middle concentration treatments induced beneficial traits with comparatively less toxicity. The most beneficial traits obtained through the treatment was the production of

polyploidy in M_2 generation. The term polyploidy was discovered in 1907 [7]. Polyploidy can be defined as a condition with more than two sets of heritable chromosomes which is responsible for the evolution of plants [145]. Naturally the polyploids occur in approximately 70% of all the angiosperms [52, 93] due to the formation of unreduced gametes. However, they can also be induced artificially through the chromosome doubling in somatic cells.

Induction of polyploidy is dependent upon mitotic inhibition capacity of the chemicals which can be induced through various treatment processes involving plant parts like roots, apical meristems and seeds. Chromosome counting can be considered as the dependable method of determining polyploids in plant systems [92]. Induced polyploidy can be confirmed either through direct or indirect method, wherein direct method involves chromosome counting, being the tedious still precise approach [120]. Alternatively, indirect method is comparatively simpler and easier involving less time with simple screening instruments [44]. Polyploidy results in chromosomal rearrangements leading to genetic variations in the species [72] and increases the cells size by adding extra copies of genes [121]. In many plants, particularly crops, mitotic polyploidy had been induced through mutagens which leads to novel phenotypic variations. Such polyploidy involves the chromosome duplication in somatic cells producing genetic variations in the existing group of plants [83]. Such genetically evolved plants then could be stored or used in the upcoming crop improvement breeding programmes. This makes the production of polyploidy immensely important for mutation breeding and is being induced extensively in the crops. However, production of polyploidy can have various limitations also. The increased number of chromosomes due to polyploidy could be responsible for increased nucleus volume altering the stability between both chromosomes and nuclear components [30, 24, 88]. Eventually this could end up with the generation of numerous abnormalities during mitosis and meiosis. Moreover, it can disrupt the physiology and genetic stability of the plants [148].

In mutation breeding, many chemicals like Colchicine, Trifluralin, Oryzalin, Acenaphthene, 8-Hydroxyquinoline and Nitrous Oxide have been discovered to have the potential of inducing polyploidy in plants. However, Colchicine is widely used to produce polyploidy owing to its high mitotic inhibition properties. While the other chemicals are not as effective as colchicine and are not in common use [123, 85, 69, 89, 11]. Colchicine is a toxic chemical derived from the seeds of *Colchicum autumnale* (Autumn crocus) which is widely used for inducing polyploidy in plant breeding. Low doses of colchicine ensure the safe induction of mutation in the plants with less toxicity [119].

Nevertheless, in the present experiment, Monosodium Glutamate was used with a view to determine its potential in inducing polyploidy, wherein *Urginea indica* Kunth after treatment was reported to generate polyploidy with double sets of chromosomes which was diploid in control being 2n=20 chromosomes. Besides, multiple chromosomal aberrations were also observed after Monosodium Glutamate treatment which could be considered as the frequent mutation produced in the target plants [50]. These are the most dependable indices for assessing the mutagenic potency of mutagens. The generation of polyploidy after treatments might had increased the marked area availability for Monosodium Glutamate in the cells, ensuing the increased frequencies of chromosomal aberrations with the increased rate of polyploidy. The chromosomal aberrations induced by chemicals are thus, considered to have a vital role in the crop improvement aid. However, many chromosomal aberrations are not beneficial which can be eliminated selecting the beneficial one [60].

The effectiveness and efficiency of Monosodium Glutamate as a potent mutagen for polyploidization in *Urginea indica* Kunth had been established through the present experiment. Induction of polyploidy in the root tip cells of the bulb of *Urginea indica* Kunth could be attributed to the fact that it would result in the increased size of the bulbs. It should be noted that the bulbs of the *Urginea indica* Kunth holds high therapeutic value and increase in the size of the bulbs can be the desirable trait expected from the controlled and planned use of Monosodium Glutamate in the laboratory and then in the field. However, Monosodium Glutamate so far has not been used as a mutagen to produce polyploidy in plants and we completely lack with any data to test its potency. Therefore, further investigations are needed to be endeavored for supporting the hypothesis of using Monosodium Glutamate as a promising mutagen in inducing polyploidy. Moreover, future investigations should include diverse populations with varied doses and time duration to get the desired results through the selection of precise experiment design.

CONCLUSION

The selected food additive, Monosodium Glutamate could be used as a promising mutagen to induce genetic variations in the selected plant, *Urginea indica* Kunth. Its effectiveness and efficiency in the genotypic traits of *Urginea indica* Kunth in two subsequent generations, viz. M_1 and M_2, in the present study confirmed its potency in inducing polyploidy with wide range of genetic variations. The obtained mutants of *Urginea indica* Kunth

could either be directly used as a new variety or it could be subjected to hybridization programmes. However, the mutagenic effectiveness and efficiency of the Monosodium Glutamate needs further studies with precise knowledge of time, dose and environmental conditions to enhance its potential.

DATA AVAILABILITY

Not applicable

FUNDING

Not applicable

ETHICAL STANDARD

Not applicable

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Figures



Figure 1

Photographs of Hysteranthus variety of Urginea indica Kunth

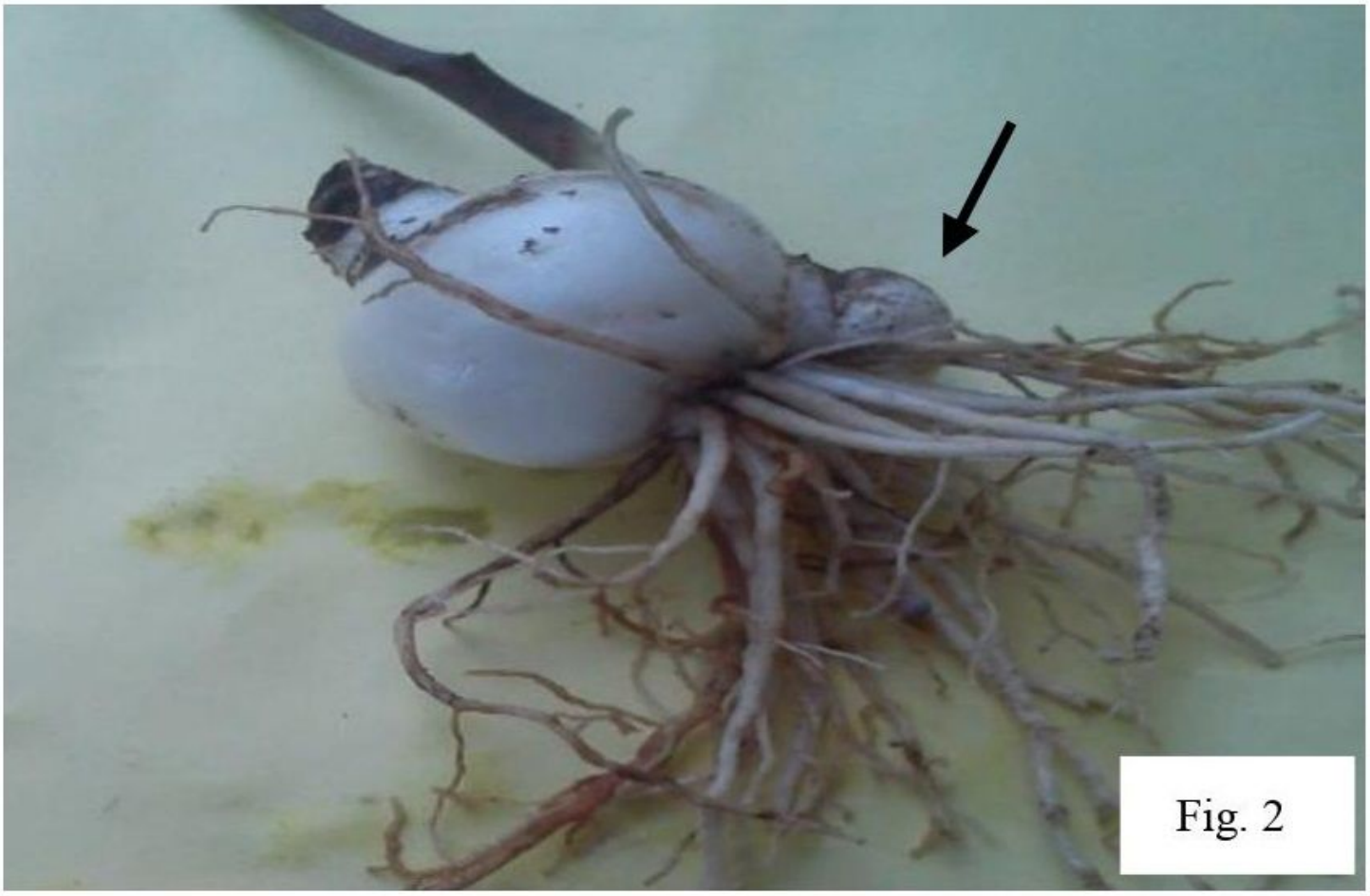


Figure 2

Photographs of bulbs of Urginea indica Kunth

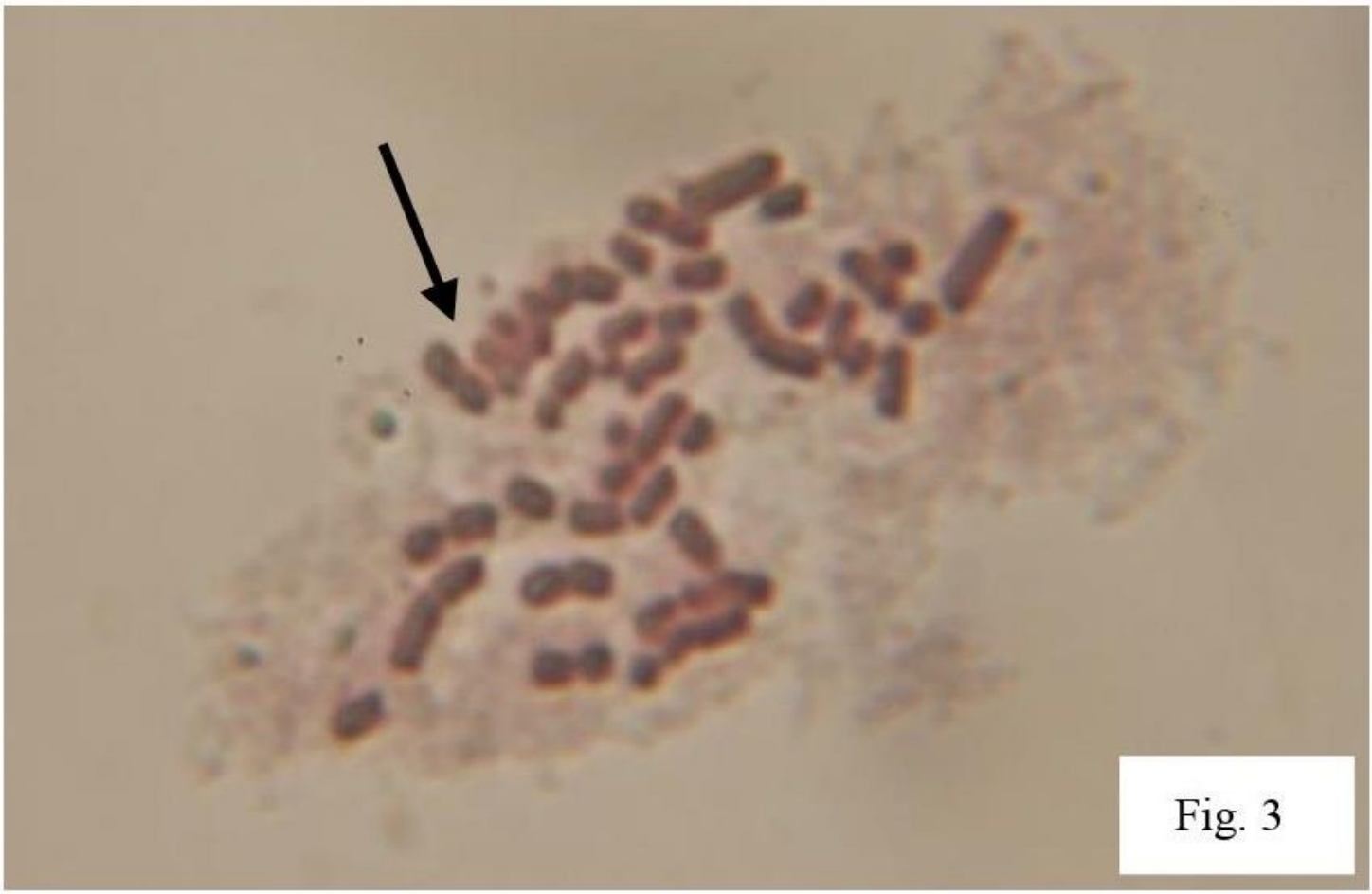


Figure 3

A polyploidy cell, nucleolus at metaphase, diagonal metaphase

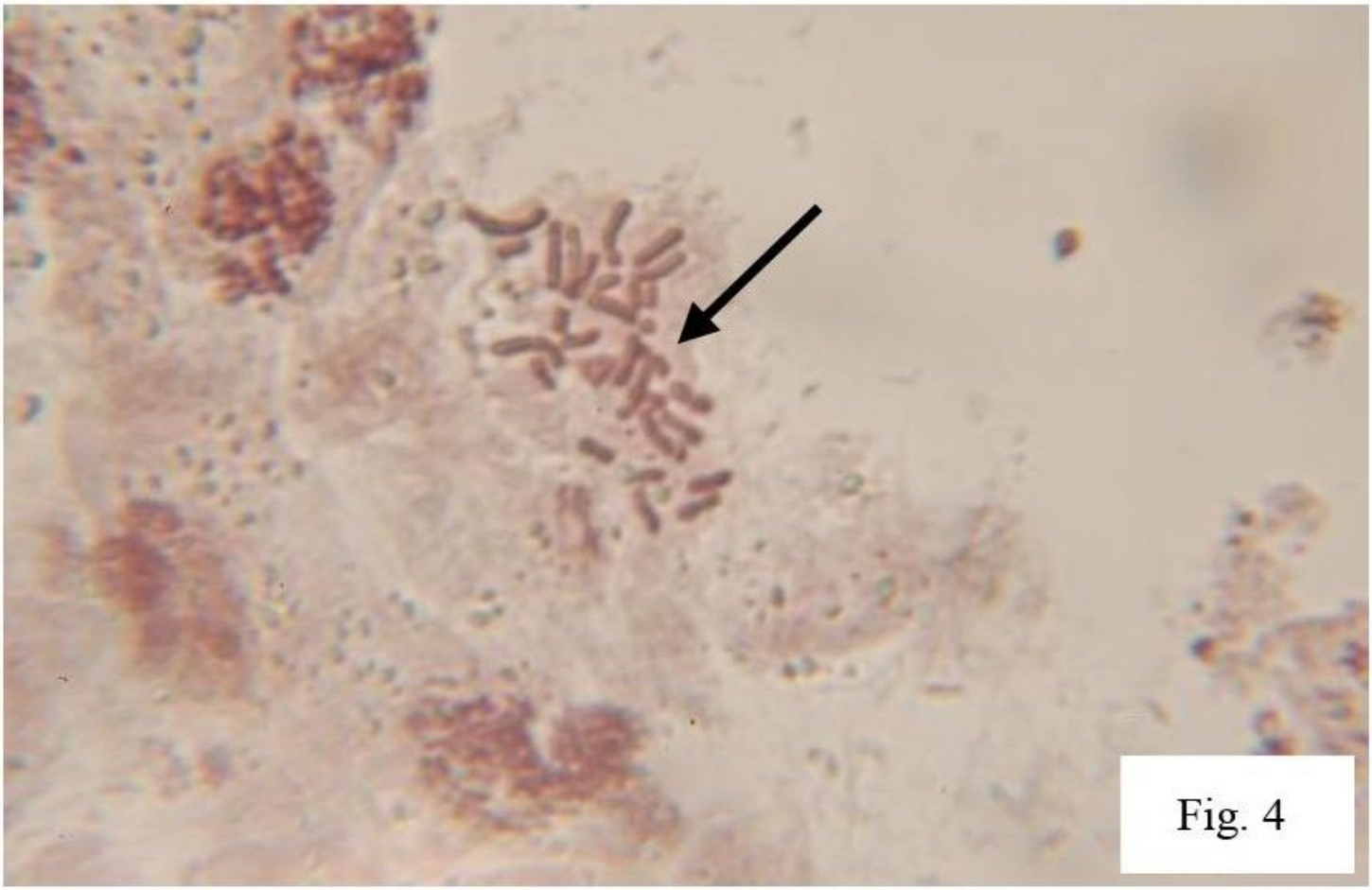


Figure 4

A polyploidy cell, nucleolus at metaphase, diagonal metaphase

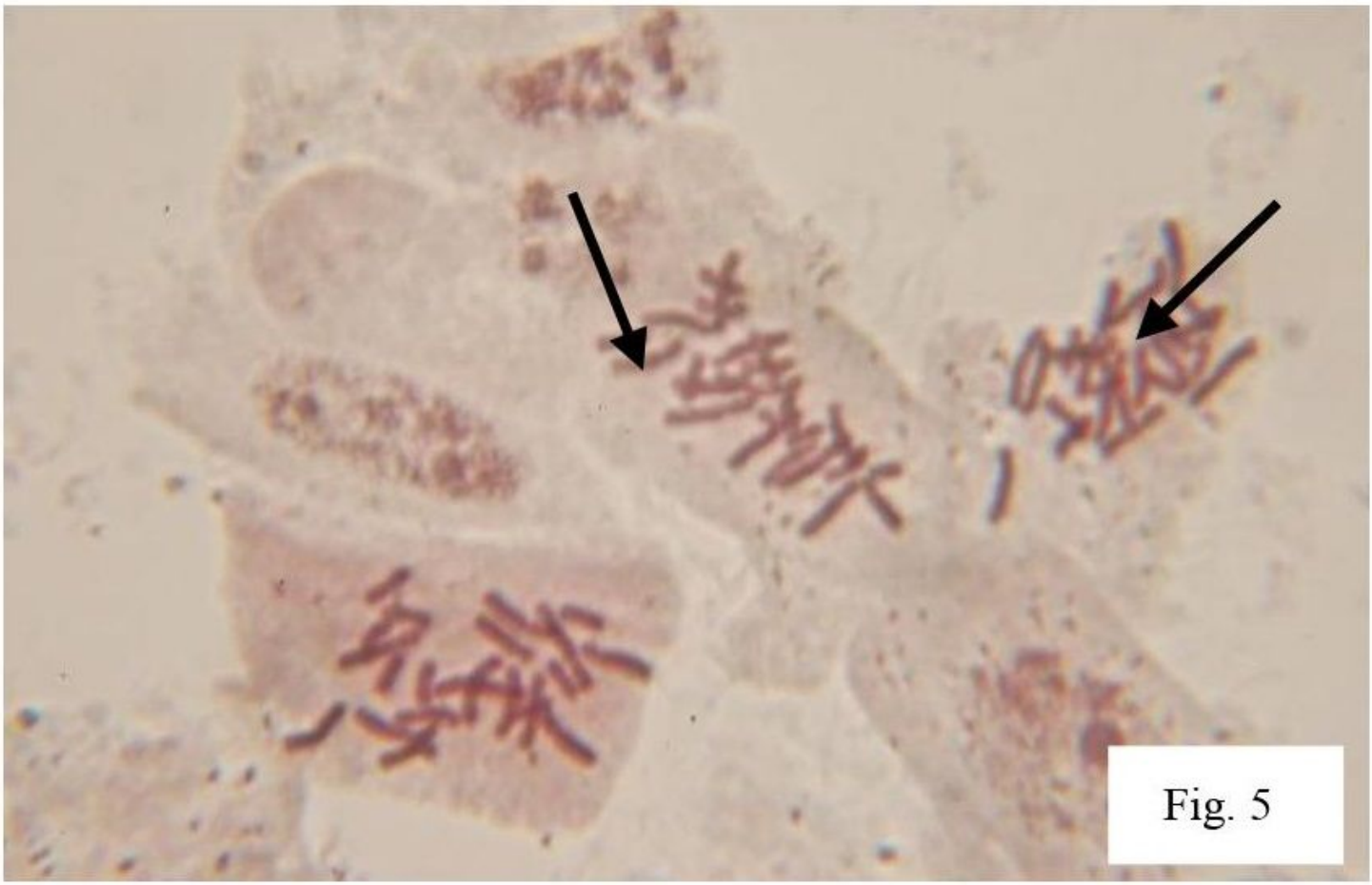


Figure 5

Cell showing tropokinesis at metaphase

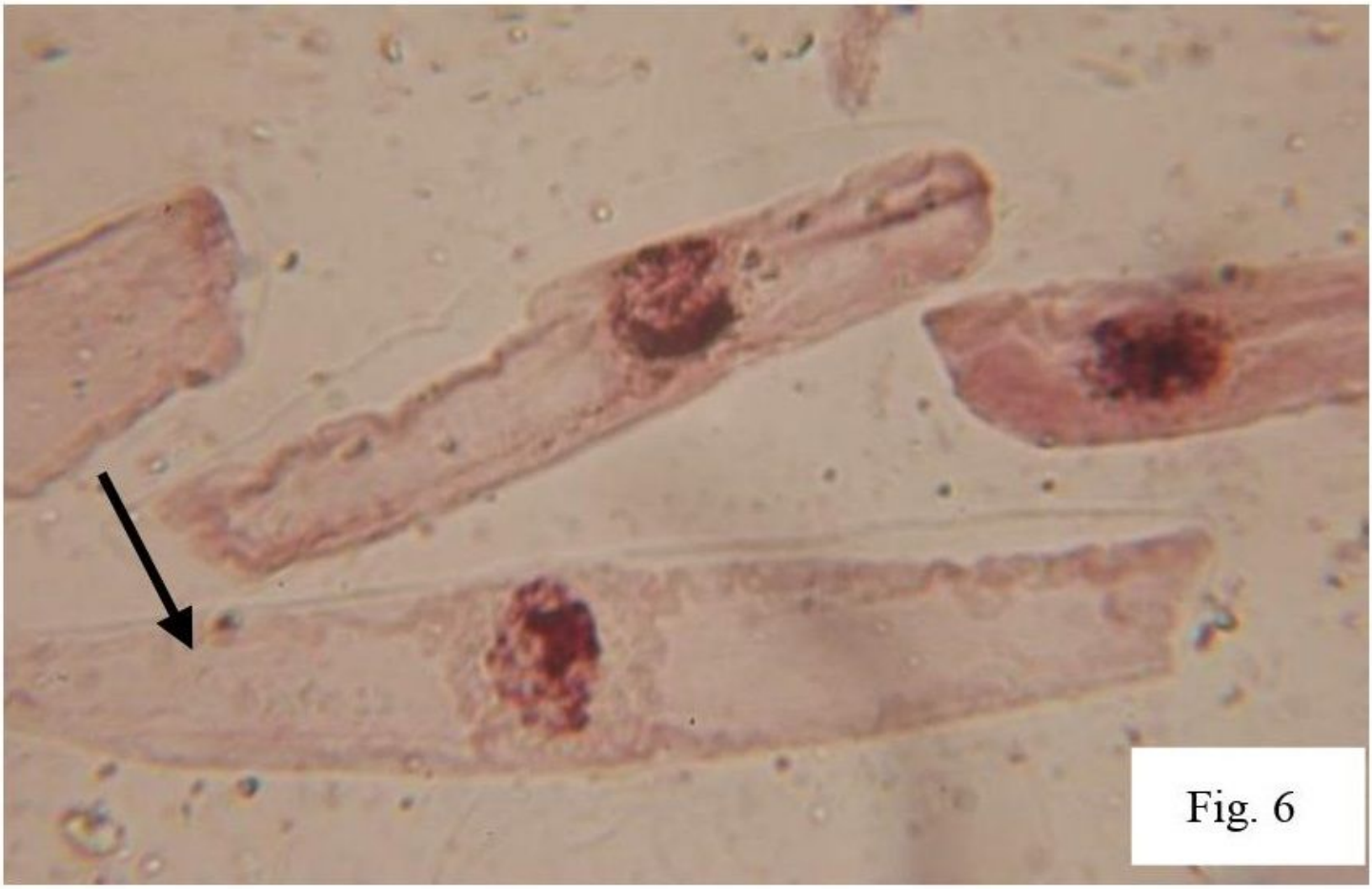


Figure 6

Elongated cells

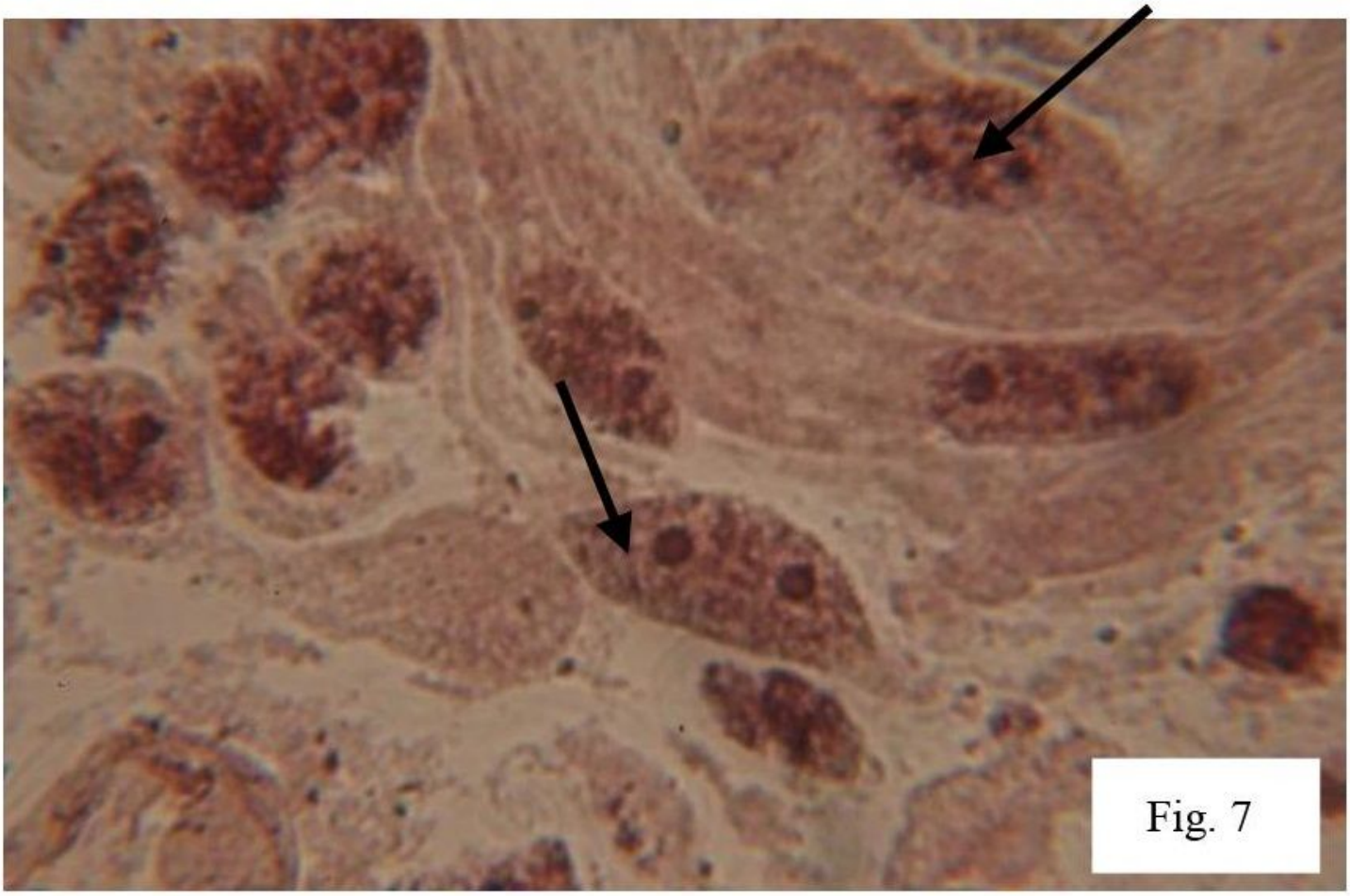


Figure 7

Binucleate cell



Figure 8

Multinucleate cell



Figure 9

Translocation ring

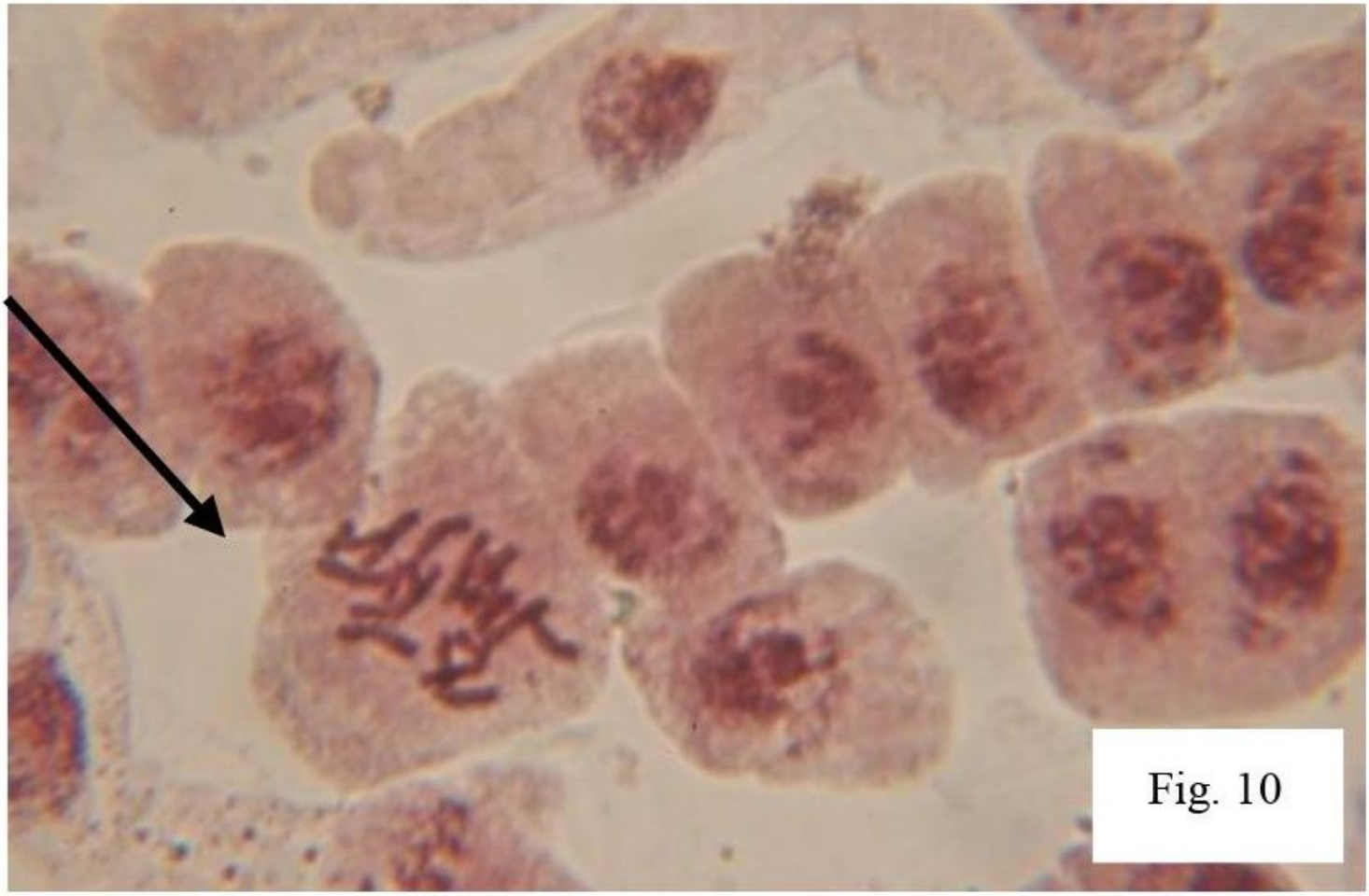


Figure 10

Cell showing precocious movement of chromosomes at metaphase

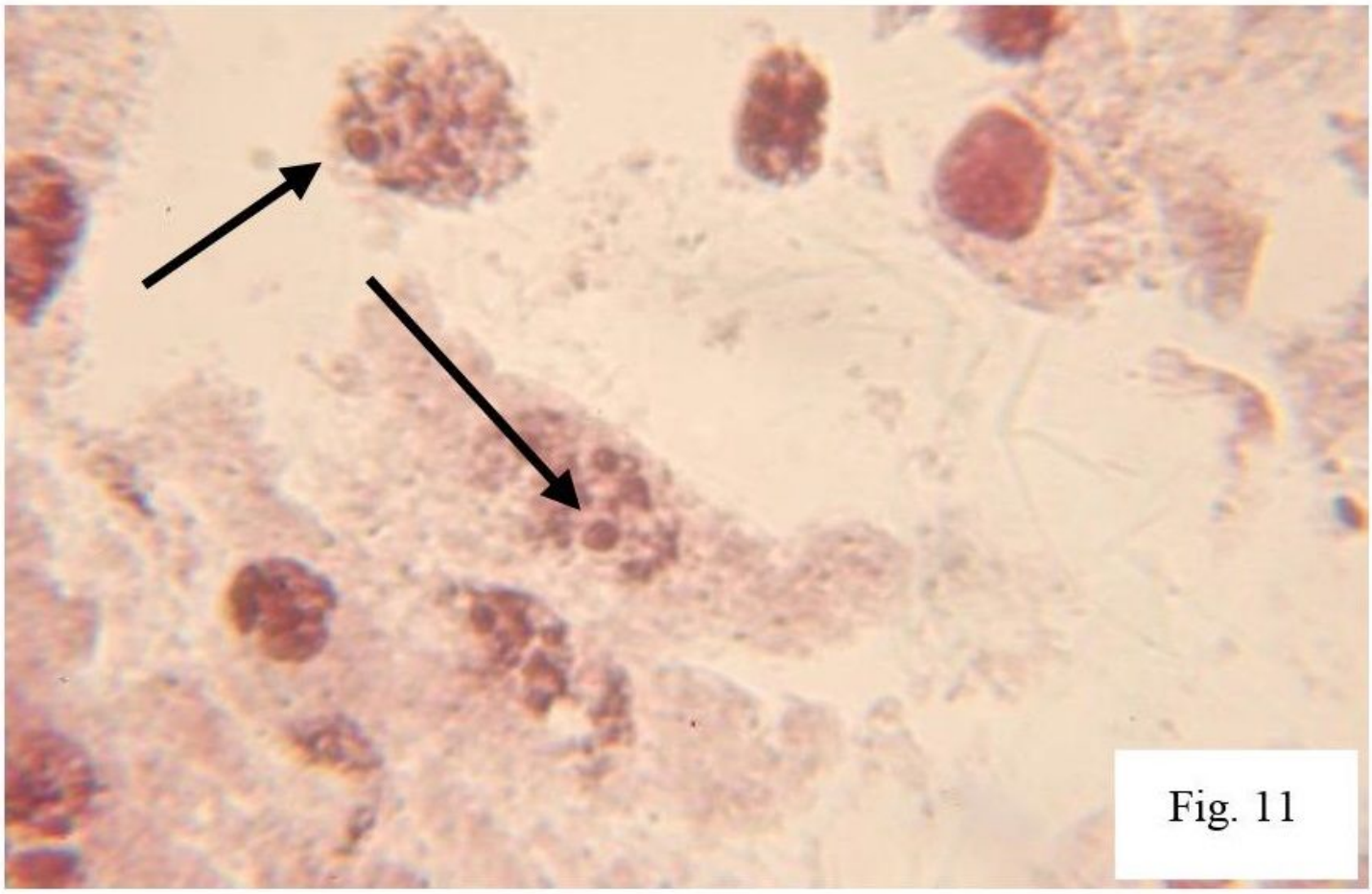


Figure 11

Cell showing micronuclei

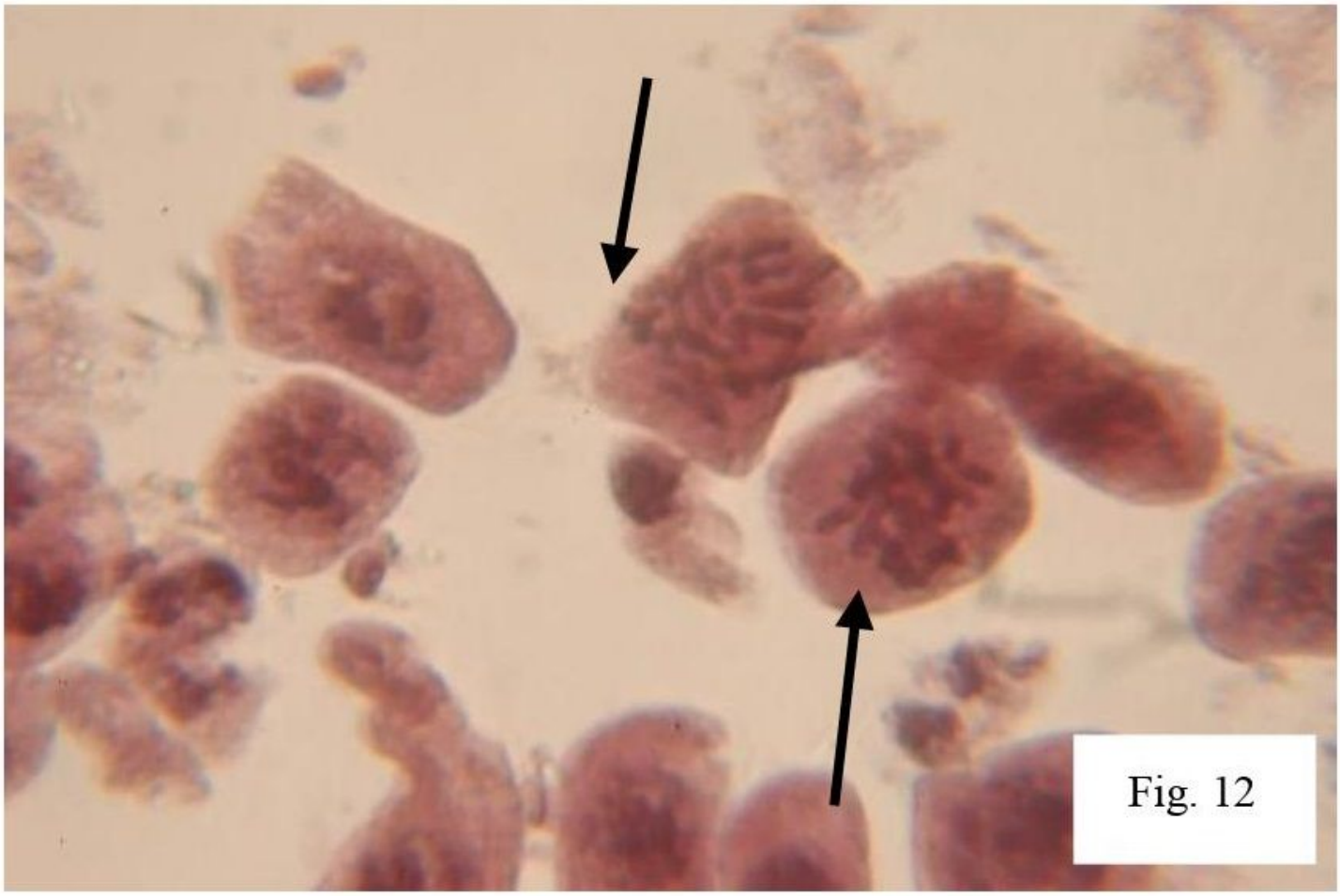


Figure 12

Cell showing sticky metaphase with precocious movement of chromosome

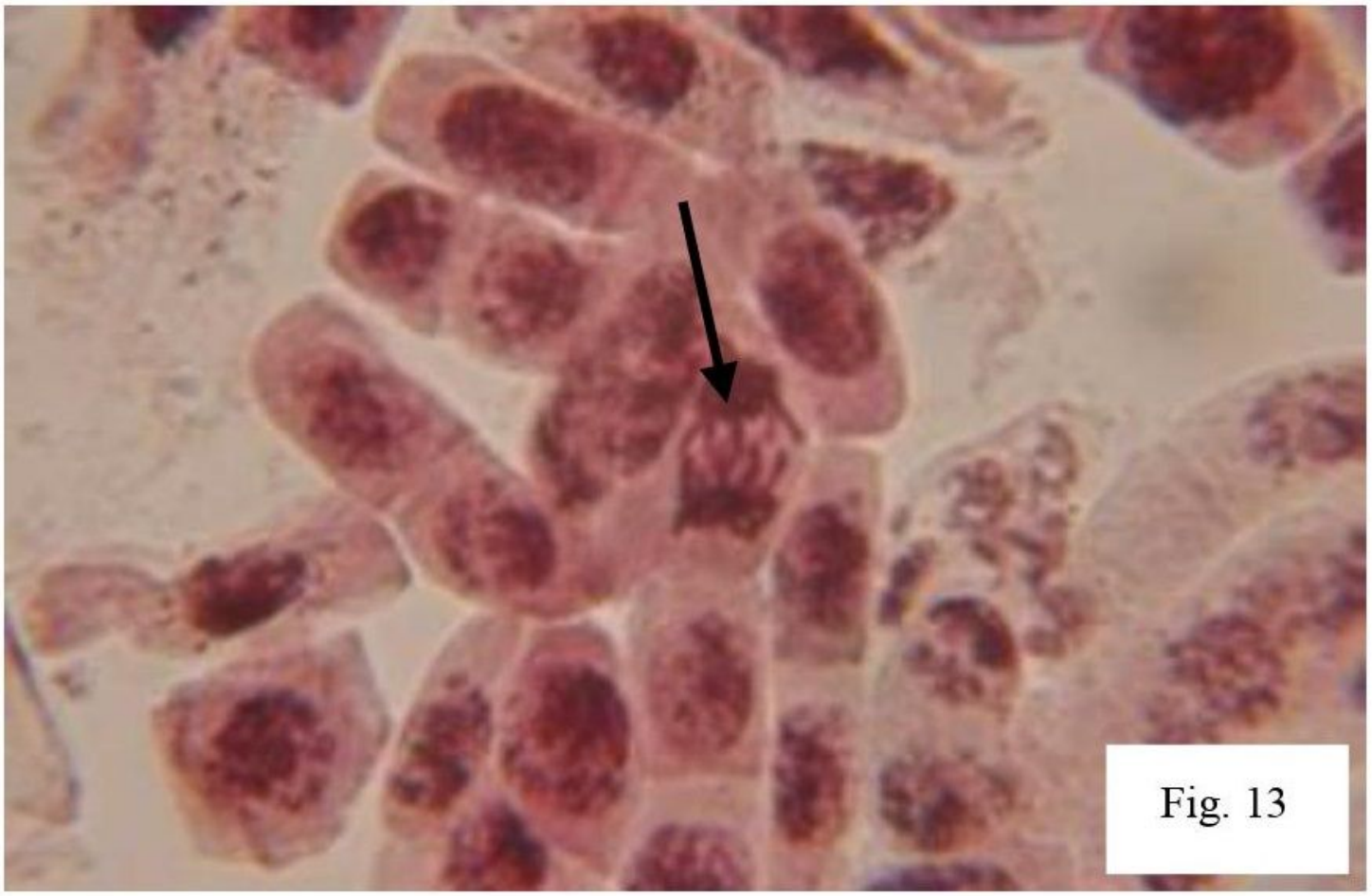


Figure 13

Cell showing disturbed anaphase

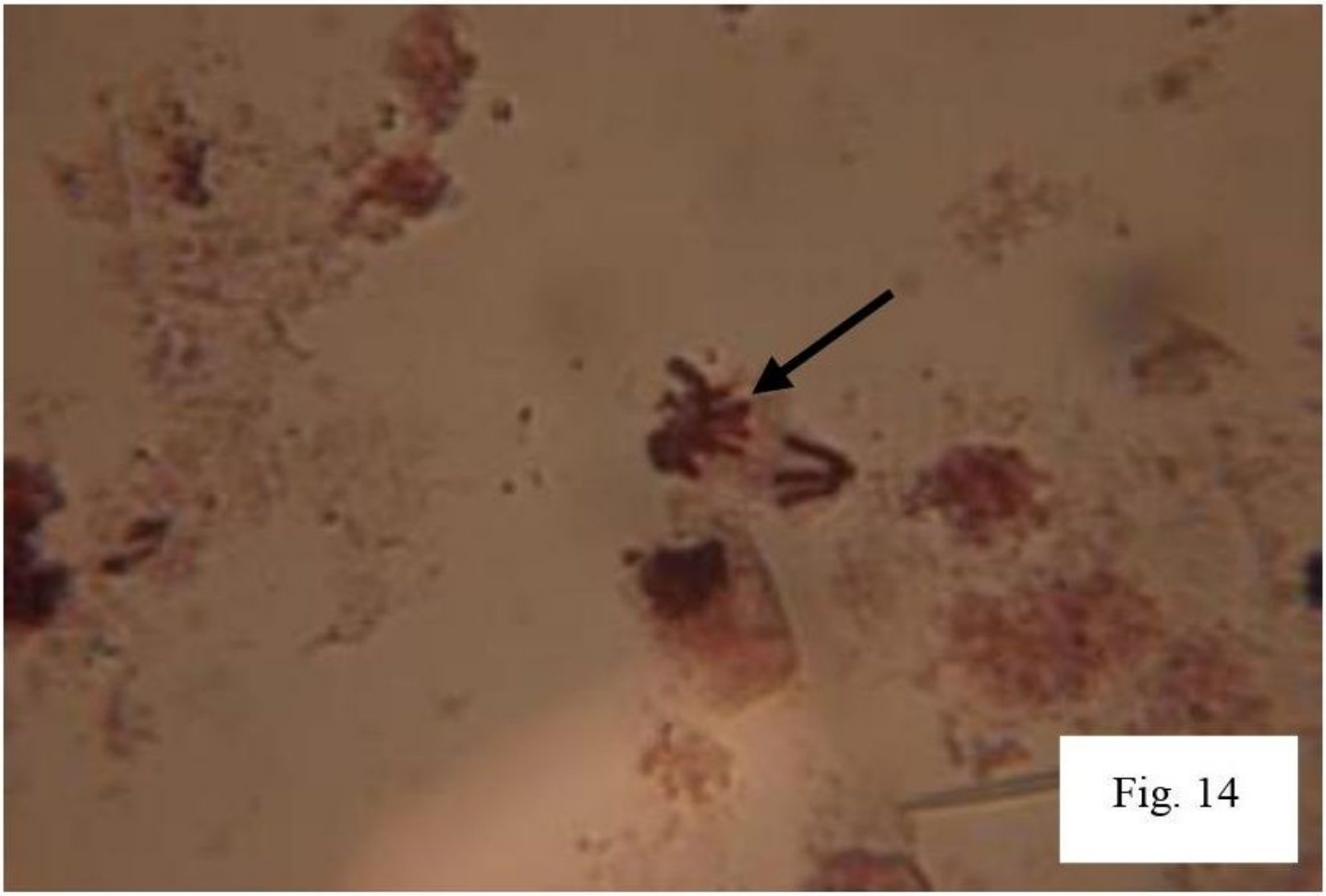


Figure 14

Cell showing unequal separation at anaphase