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The impact of contaminated water with pharmaceutical hazards on the *Vicia faba* plant cell's components and mitosis behavior

Mariam Alketbi¹, Rania Ali^{2*} and Abdullah A. L. Mutery¹

Abstract

Introduction Paracetamol may have its potential risk to ecosystem when pollute water resources around the hospitals and medicines' factories areas. Water recycling systems are admitted for irrigation purposes to cover the high needs in water demands. The pharmaceutical hazards in the recycled water should be investigated for its effect on plants' stability and performance.

Method This work carried the cytogenetics and cytological studies to visualize the plants performance under paracetamol stress in four concentrations (0, 6, 12.5, 25 and 50%) in two treatment times 3 & 6 h. On the other hand, the recovery experiment for 24 h after treatment was carried to precise the plant cure status. The study is also supported with TEM (transmission electronic microscope) descriptive investigations to many vital cytoplasmic organelles that affect the plant responses, adaptation, energy level and all metabolic process.

Results Despite that, the higher used concentration has its cytotoxic and turbo-genic impacts yet, it did not represent any mutagenic threat. It exerted an oxidative stress, chromosomes aberrations (stickiness and disturbances) and genotoxicity (fragmentation) in *Vicia faba* plant cells. The recovery treatment demonstrated the flexibility of plant detoxification systems to survive and to retain some of its fitness after the influencer disappears.

Conclusion Paracetamol affected *Vicia faba* plant cells in a dose-dependent manner and induced oxidative stress and genotoxicity in *Vicia faba* plant cells. Therefore, it is important to monitor and regulate the presence of paracetamol in the environment and to develop effective strategies for their removal or degradation from the recycled water as a previous step before being used in plants irrigation.

Keywords Transmission electronic microscope, Mitosis, Oxidative stress, Paracetamol

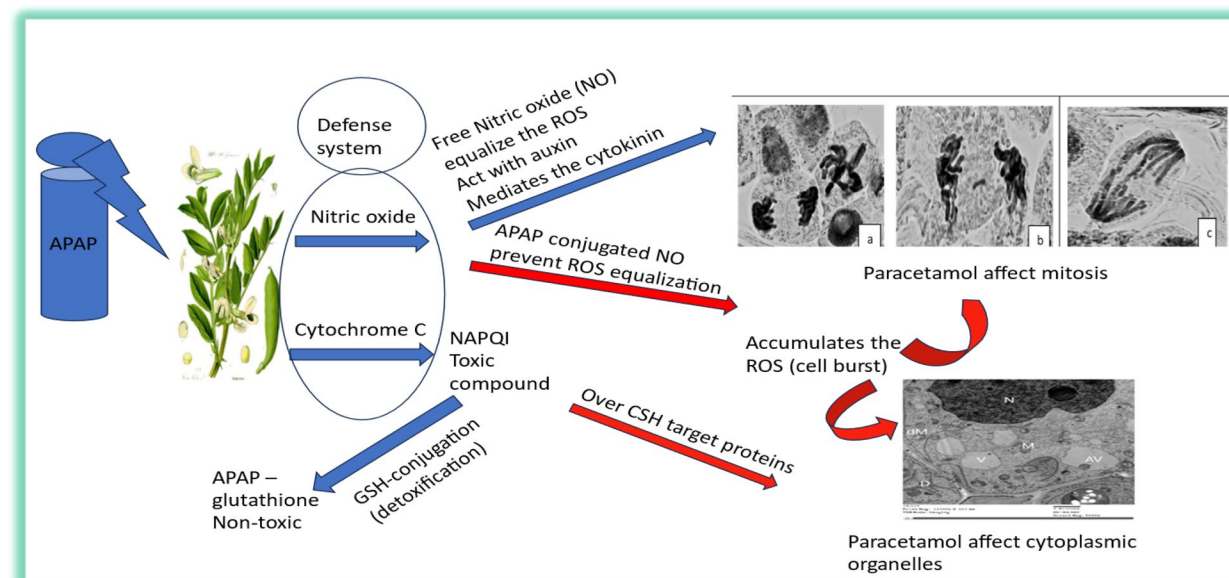
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Graphical abstract



1 Introduction

Water treasure is really concerned and considered in our nation area, recycling the waste water is a must to cover the increasing needs for water which is accompanied with limited water available supplies. Sharps, infectious waste and pathological waste water are all subject to waste separation, and the purified water may be reused in irrigation.

The primary causes of the presence of pharmaceutical waste in the environment include improper waste disposal of used containers, unused medication or livestock feed, as well as manufacturing processes in a variety of public locations, including the surroundings of hospitals and pharmaceutical manufacturing facilities, the roadside or city corporation dustbin and homes [1].

According to an analytic studies of waste water containing chemicals under the Medical Waste Management (MWM is a system used in the UAE) program, it was assessed that hospitals produce an average of 1.95 kg of medical waste per bed per day, ranging from (0.2) to (4.5) kg/bed/d) in other hospitals [2]. The complexity and toxicity of hospital waste make markedly differences from other residential waste, as it contains standard human excretion wastes, discharges from technical equipment and laboratories' drugs and metabolites such as disinfectants and some microorganisms,

which are frequently characterized by the presence of antibiotic resistance due to their "infectious" nature [3].

In aquaculture and agriculture, drugs that are used to prevent and treat disease are a significant source of pollution [4, 5]. Widespread use of veterinary medications, including antibiotics, hormones, antifungal, antiparasitic, anesthetic and anti-inflammatory, has its impact on non-target species like plants [6]. Additionally, a problem that is becoming increasingly prominent is the pollution of the soil environment with pharmaceutically active substances (also known as PACs) [7]. Inadequate water chemical disposal planning and open dumping of hospital wastes into hospital surroundings and roadsides have resulted in the greatest public health risks [8]. Human pharmaceutical residues in the environment have been extensively studied in recent years, with an emphasis on invertebrates and higher organisms in aquatic environments [9]. There are a few studies on human and veterinary drug uptake experiments in higher plants, but there is less information on metabolism and effects inside plants [10].

In several research works, many pharmaceutical compounds had approved for their genotoxic and cytotoxic effects on plants. It may interact with plant metabolisms [11], accumulate in plant, phytotoxic the plant [12], elevates the ROS level [13].

Also, many crop plants were investigated for their response to the pharmaceutical hazards in irrigation water [13, 14].

Paracetamol (acetaminophen) (formula $C_8H_9NO_2$) represents one of the most widespread pharmaceuticals found in surface waters and sediments around the world. Although wastewater treatment plants can remove up to 80% of the paracetamol in water, the remains can negatively impact the receiving environment [15].

Paracetamol is common analgesic medicine that has been proved to have genotoxic effects on a wide range of organisms, including plants [16]. It is available over-the-counter in several countries and is frequently used to relieve pain and fever [17]. When taking according to instructions, paracetamol is usually regarded as safe and efficient, while excessive usage can have negative consequences such as liver damage. The mechanism through which paracetamol works is to prevent the body from producing prostaglandins, which are molecules that induce pain and inflammation [18]. It is quickly absorbed from the digestive system and is processed by the liver. If unchecked, liver damage or even failure brought on by a paracetamol overdose can be deadly [19]. Within 24 h of use, the bulk of paracetamol is eliminated in the urine and be received to the environment. However, adverse environmental effects of paracetamol have not been extensively studied. Managing the hospital's drain water that contains toxins, chemicals and potentially dangerous medications like paracetamol (acetaminophen/APAP) is a critical concern for the environment, animals, plants and public safety [20, 21].

Faba bean is a great plant model for cytogenetic and cytological studies evaluation for the environmental toxins, chemical and physical hazardous substances due to its features [22].

Mitosis apparatus is the system responsible for plant growth and renewal of the ex-damaged cells; this system is controlled by growth hormones and enzymes in addition to the potential energy level of the divided cells. Normal mitosis reflects the healthy status of the plant and reflects the direct effect of the examined material on the plant cell cycle and the normal rate of growth [23].

This study designed to evaluate the effects of paracetamol on the vegetative stage of faba using a series of toxicity experiments on cytogenetic and cytological levels in order to find out the effect of paracetamol as a pharmaceutical hazard in irrigation water on the *Vicia faba* plant performance during vegetative stage.

2 Materials and method

All the conducted experiments were carried out on *Vicia faba* seeds (Giza 716) were purchased from Egypt's National Agricultural Centre.

Chemicals:

- Tested material

Acetaminophen (paracetamol) powder, manufactured by Sigma-Aldrich (USA), diluted in 1 ml of ethanol to assure complete dissolve in water and generate suspensions in concentrations. Different concentration was prepared from this suspension (6, 12.5, 25 and 50%) and used in the study for their impact after direct treatment.

- Chemicals for cytogenetic studies

Carnoy's fixative: 1-part glacial acetic acid: 3 parts of chloroform: 6 parts of absolute ethyl alcohol [24].

- Chemicals for TEM studies

The chemicals for cytological studies with transmission electronic microscope were prepared according to [25] with some modifications.

2.1 Methods

Faba seeds were submerged in humus for 12 h, germinated on wet filter paper sheets in beakers with distilled water in bottom.

The cytogenetic and cytological researches were carried on the meristem cells at the root tips in the main roots nearly 2 cm in length after treatment with different concentration of paracetamol for 3 & 6 h and 24 h of recovery after treatment.

2.2 Squashing and observation of slides

The root tips were fixed for 24 h in Carnoy's solution at 4 °C, rinsed with distilled water and transferred to 70% alcohol at 4 °C. Slides were made using the acetocarmine squash technique guided with [24], and observations were made at a magnification of under 40×. Biological microscope from the Motic BA210 series was used to take the photos.

Three thousand cells from each group of *Vicia faba* were studied on a slide that covered 250 cells, for a total of 1000 cells per slide. The fraction of dividing cells was used to construct the mitotic index; SPSS software was used to carry on the data statistical management. Additionally, chromosomal abnormalities, fragments, aberrant orientation, lagging chromosomes and other conditions were looked for on the slides.

- Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) descriptive studies were carried out at the laboratory CURP (Cairo University Research Park, Faculty of Agriculture). The roots of *Vicia faba* seedlings (Var. Giza 716) were treated with the test materials for 3 h and then immediately fixed in 5.0% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3). Post-fixation was done using 1% osmium tetroxide in 0.1 ml sodium cacodylate buffer. Dehydration was carried out using a series of increasing concentrations of ethanol, starting from 40% and ending with absolute ethanol. Clearing was done with propylene oxide for 30 min, followed by infiltration with propylene oxide—Araldite Mixture (1:1) for 30 min.

The samples were then embedded in freshly prepared Araldite embedding mixture in labeled compartments and polymerized (hardened) to form plastic blocks suitable for sectioning. Semi-thin sections of 1 μ m were initially cut using an LKB ultra microtome [ULTRA CUT-UREICH-JUNG], mounted on glass slides and stained with toluidine blue for light microscopic examination before the final trimming around a section comprising the endometrial epithelial lining and the stromal tissue.

Ultra-thin sections were then mounted on metal copper grids, stained with uranyl acetate for 30 min followed by lead citrate for 15 min and examined using a transmission electron microscope (JEM-1400 TEM) at the desired magnification. Images were captured using a CCD camera model AMT, optronics camera with a

1632 $\times$ 1632 pixel format as a side mount configuration, utilizing a 1394 firewire board for acquisition.

3 Results

The direct treatment with paracetamol induced a fluctuated increase in the plant mitotic index over the non-treated plants (control). The 24 h of recovery after treatment showed a fluctuated decrease in the mitotic index relative to the treatment (except after 25% for 3 h.) and relative to control. The highest mitotic index scored after treatment with 6% of paracetamol to reach 22.53%. The least mitotic index was 14.43% scored after treatment with 25%. Expanding the treatment time to 6 h showed an increase in the mitotic index after all the used concentration over control to reach the maximum value 25.90% after 25%. The recovery treatment announces the 25% of treatment the most mitotic enhancer to score 16.87 (Fig. 1).

Worth to mention that, only the 24 h of recovery after 6 h of 50% of treatment showed an increase in the mitotic index relative to the treatment and control.

Concerning the phase index on the level of 3 h of direct treatment, the means of phase index showed an increase in the prophase index and metaphase index at 6 & 12.5% paracetamol concentration to reach (90.71 & 7.15) and (88.73 & 8.62), respectively; on response the anelophases, this means that about (97.86 & 97.35%) of divided cells were accumulated at these stages and cannot pass through to next step of chromosomes division. This was reversed after treatment with the two higher concentration 25 & 50% of paracetamol as there was a

*List of figures

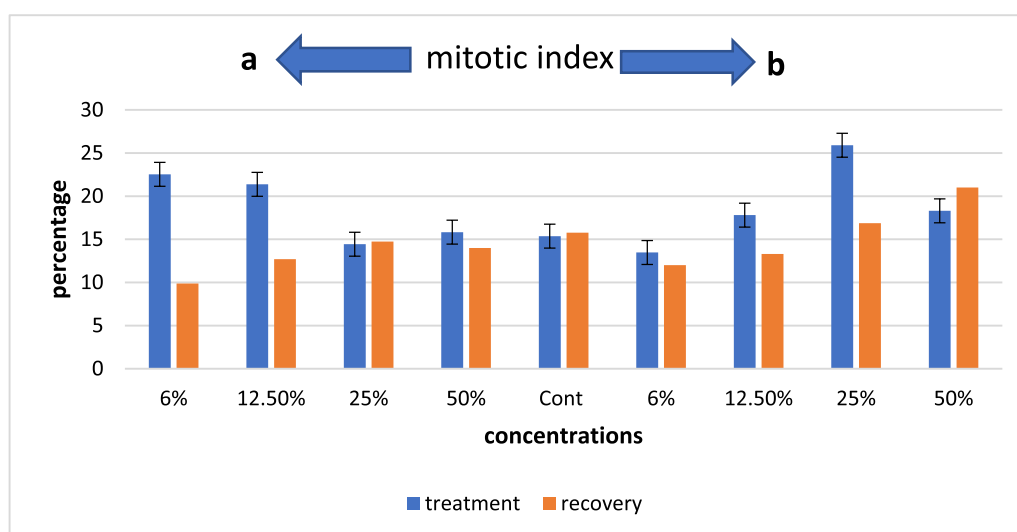


Fig. 1 The effect of direct treatment with paracetamol for 3 h (a) & 6 h (b) and recovery treatment for 24 h on the mitotic index of *Vicia faba* root tip meristems

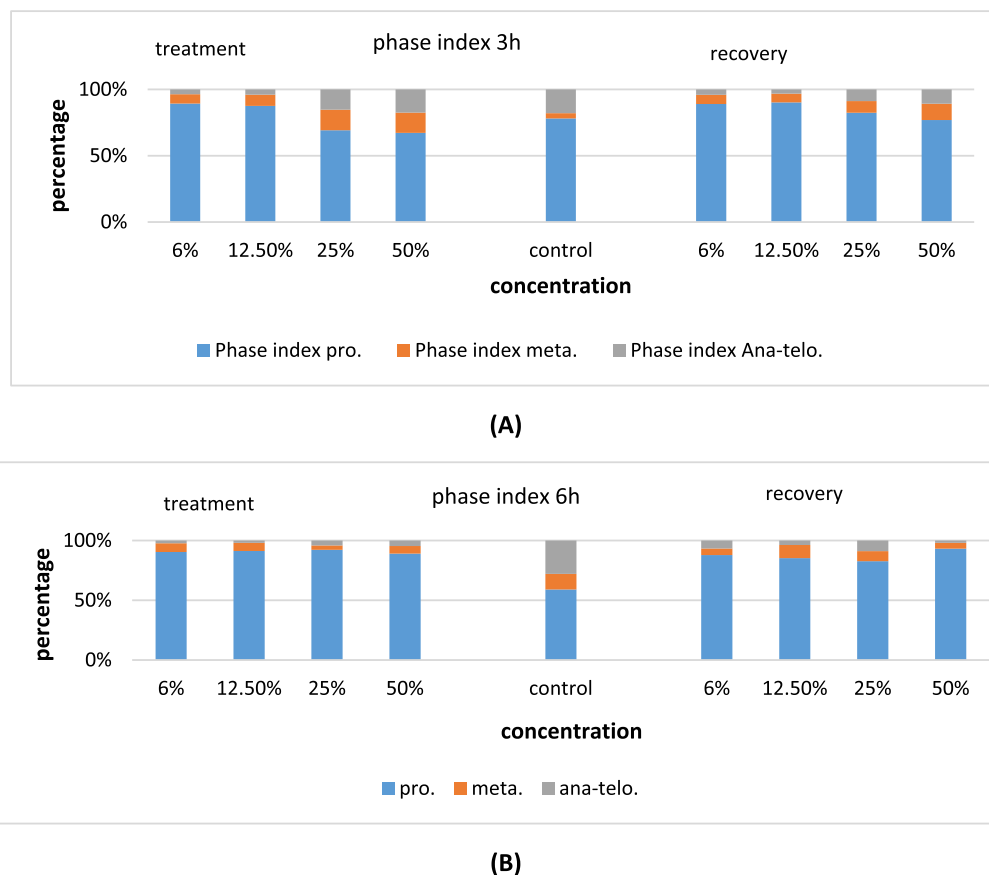


Fig. 2 The effect of direct treatment with paracetamol for 3 h & 24 h recovery treatment (A) and for 6 h & 24 h recovery (B) on the phase index of *Vicia faba* root tip meristems

remarkable decrease in the prophase (to reach 74.29 & 71.63%, respectively) and a remarkable increase in the metaphases (to reach 16.68 & 16.30%) and telophases (to reach its highest value 18.60% after 50% even higher than control 17.94%) (Fig. 2).

Same was noticed after the recovery treatment with only difference that the prophase indexes after the two higher concentrations showed an enhancement in the prophase index to exceed the control after 25% and to equal the control after 50%.

Expanding the treatment time to 6 h showed that all the used concentrations showed a remarkable increase in the prophase over control by percentage nearly 50% of its value. This increase came on response to ana-telophases nearly by same percentages relative to control, the recovery experiment showed an increase in the ana-telophases after the 6, 12.5 and 25% of paracetamol, while the recovery experiment after the 50% of paracetamol did not show any enhancement which reflect the irreversible effect of this concentration on the phase index.

On the level of the abnormality distribution: Data in Tables 1 and 2 reveal the direct treatment with

paracetamol for 3 & 6 h which scored the maximum percentage of abnormalities in prophase. The only difference is that 3 h of treatment induced a concentration-dependent percentage of abnormalities to reach 18.78% after treatment with 50% paracetamol; with the exception after treatment with 12.5% of paracetamol which scored 9.55% the lowest among treatments. While 6 h induced fluctuated percentage of abnormalities reach 3.59% after the 6% paracetamol. Three hours of treatment induced abnormality percentage on metaphases which were significantly increased relative to the control in the all used paracetamol concentrations. Abnormalities were increased in incidence relative to the control after 6, 25 and 50% of paracetamol and were lowered after the 12.5% paracetamol. Treatment for 6 h induced fluctuated percentage of abnormalities in metaphases to reach 3.59% after the 6% paracetamol.

The scored percentage of abnormal mitosis after the recovery experiment The recovery experiment carried after 3 h. of direct treatment showed a little improvement in the plant performance after treatment by lowering all the abnormality percentages relative to the treatment, yet

Table 1 Percentage of abnormal mitosis and the abnormality distribution in percentage in different mitotic phases; relative to the scored mitosis in *Vicia faba* root tip meristems after 3 h & 6 h of direct treatment with paracetamol (a) and after the 24 h of recovery after treatment (b)

Treatment	% abn. mitosis	%abn in Mitotic phases 3 h			% abn. mitosis	%abn in Mitotic phases 6 h		
		Pro	Met	Ana-telo		Pro	Met	Ana-telo
control	13.10	8.41	0.88	3.80	11.83	7.86	1.46	3.51
(a) Direct paracetamol								
6%	27.45	16.10	5.71	4.42	26.44	21.96	3.59	0.89
12.5%	15.02	9.55	3.43	2.03	20.09	16.04	3.23	0.81
25%	32.01	17.81	6.81	7.39	21.57	17.86	1.64	2.07
50%	32.74	18.78	7.43	6.53	21.26	16.09	3.16	2.01
(b) 24 h recovery after treatment								
6%	18.44	13.73	2.80	1.91	11.54	8.78	1.25	1.51
12.5%	13.92	10.20	2.58	1.14	22.38	15.91	4.86	1.61
25%	20.84	14.74	4.28	1.82	15.85	11.49	2.59	1.76
50%	20.74	13.40	3.65	3.69	16.51	12.90	2.11	1.50

Table 2 Percentage of different types of abnormality relative to the abnormal mitosis and its distribution among phases in means in *Vicia faba* root tip meristems after the 3 h of direct treatment with paracetamol (a) and after the 24 h of recovery after treatment (b)

Treatment	% Of different types of abnormality											
	Chromatin liquefaction				Spindle disorder				Structural disorder			
	Stickiness				Disturbances				Fragmentations			
	Pro	Met	Ana-telo	Stick	Pro	Met	Ana-telo	Dist	Pro	Met	Ana-telo	Frag. & break
Cont	3.33	0.00	3.66	34.95	9.33	1.33	2.33	64.95	0.0	0.00	0.02	0.01
(a) Direct paracetamol												
6%	14.33	5.7	6.7	46.8	21.0	6.0	2.3	52	0.0	0.22	0.50	1.26
12.5%	3.33	2.7	2.7	28.8	15.3	4.3	1.7	71.1	0.0	0.00	0.03	0.1
25%	11.66	4.7	5.7	47.4	14.3	5.0	5.0	52.5	0.0	0.01	0.01	0.04
50%	16.00	7.3	6.7	57.3	14.3	4.0	4.0	42.7	0.0	0.00	0.01	0.02
(b) 24 h rec. after treat												
6%	10.33	0.7	0.0	44.6	8.0	3.0	0.0	44.6	0.0	1.00	1.67	10.83
12.5%	2.66	0.7	1.0	31.6	7.7	1.7	0.0	68.2	0.0	0.01	0.01	0.15
25%	7.00	2.0	0.7	31.5	15	4.3	2.0	68.5	0.0	0.00	0.01	0.03
50%	5.33	1.0	4.0	35.6	13	4.0	1.3	64.3	0.0	0.00	0.01	0.03

it still rises in the abnormalities in prophases and metaphases if compared with the control. The scored abnormalities were also lowered in telophases after the 6, 12.5 and 25% paracetamol relative to the control.

The recovery experiment after 6 h. showed the positive conflict on mitosis by lowering percentages of abnormality to reach 8.78% in prophase after 6% of paracetamol concentration, yet it was still higher than control.

On the level of scored types of abnormality The paracetamol affected the chromatin liquefaction (chromo-toxic effect) (sticky chromosomes and sticky bridges) in phenomenon called (chromo-toxic effect) and chromosome

mobility (irregular prophases, prometaphases, clumped, disturbed and laggard) in what called (turbo-genic effect) and it also induced very slight effect on the chromosome integrity and the whole chromosome structure (fragments, breakage and ring chromosome) in what called (clastogenic effect) (Tables 2 and 3) (Figs. 3, 4, 5, 6 and 7).

Paracetamol concentration showed the more time-dependent effect on the spindle than it affected the chromatin viscosity on the level of the 6, 12.5 and 25%; this was reversed after the 50% of paracetamol as it showed its effect on the chromatin material more than the chromosome mobility.

Table 3 Percentage of different types of abnormality relative to the abnormal mitosis and its distribution among phases in means in *Vicia faba* root tip meristems after the 6 h of direct treatment with paracetamol (a) and after the 24 h of recovery after treatment (b)

Treat	% Of different types of abnormality											
	Chromatin liquefaction				Spindle disorder				Structural disorder			
	Stickiness				disturbances				Fragmentations			
	Pro	Met	Ana-telo	stick	Pro	Met	Ana-telo	dist	Pro	Met	Ana-telo	Frag. & break
Cont	1.33	0.00	2.00	39.0	6.00	2.00	2.00	73.21	0.0	0.00	0.00	0.00
(a) Direct paracetamol												
6%	14.0	1.66	0.00	33.8	23.7	5.33	1.66	66.16	0.0	0.00	0.02	0.043
12.5%	11.3	1.66	0.00	46.96	11.0	2.66	1.00	53.00	0.0	0.00	0.01	0.04
25%	17.3	2.33	2.00	44.8	24.3	1.33	1.66	56.53	0.0	0.00	0.00	0.00
50%	16.3	4.33	2.33	59.5	12.3	1.66	1.66	40.48	0.0	0.02	0.00	0.05
(b) 24 h rec. after treat												
6%	4.00	0.00	0.00	26.1	7.66	1.66	1.66	71.62	0.0	0.00	0.32	2.09
12.5%	4.66	1.66	1.33	28.7	14.0	4.33	1.00	72.51	0.0	0.00	0.00	0.00
25%	3.33	0.00	2.00	20.0	12.7	2.00	2.00	62.49	0.0	1.00	3.47	17.52
50%	10.7	1.00	2.00	40.18	16.0	3.33	1.33	60.76	0.0	0.00	0.00	0.00

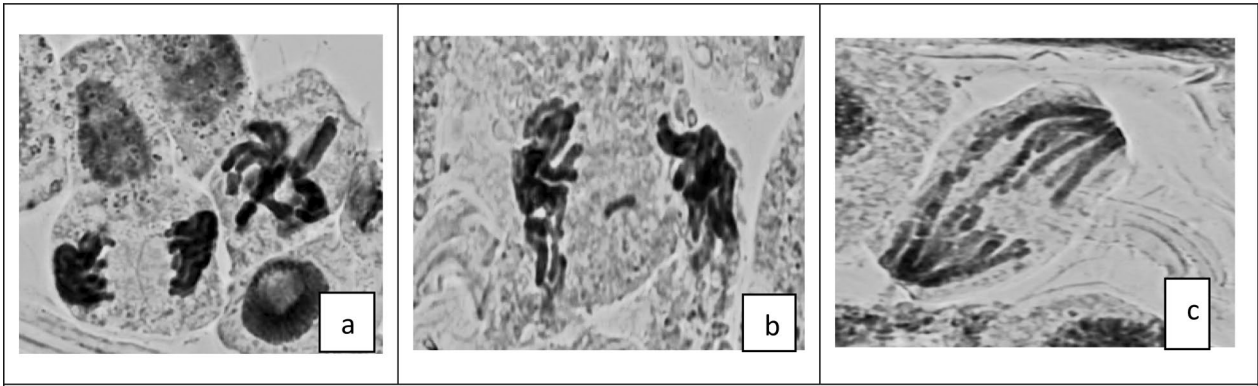


Fig. 3 The effect of 3 h treatment with 6% paracetamol on mitotic chromosomes of *Vicia faba* root tip meristems: slight sticky metaphase and sticky anaphase with microsatellite (a), sticky anaphase with laggard chromosome (b) and anaphase with fragmented chromosomes and bridges (c)

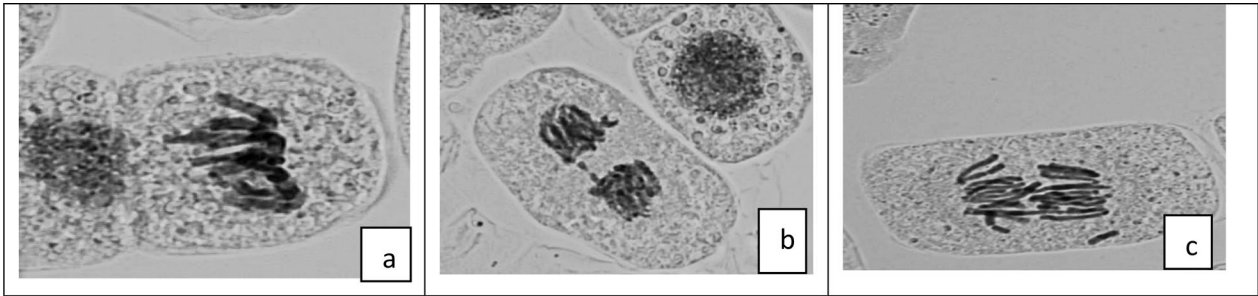


Fig. 4 The effect of 6 h treatment with 6% paracetamol on mitotic chromosomes of *Vicia faba* root tip meristems: clumped metaphase (a), anaphase with broken bridge and fragment (b) and disturbed anaphase (c)

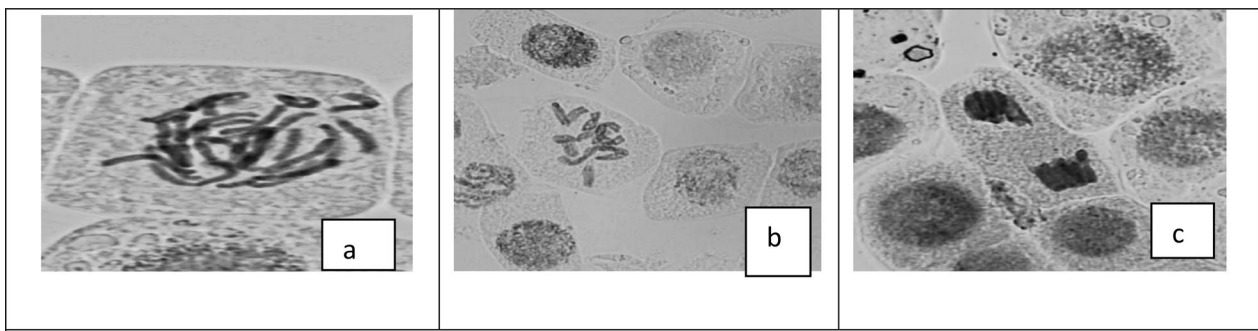


Fig. 5 The effect of 3 h treatment with 12.5% paracetamol on mitotic chromosomes of *Vicia faba* root tip meristems: irregular prophase with ring chromosome (a), disturbed metaphase (b), sticky anaphase with adhered fragment (c)

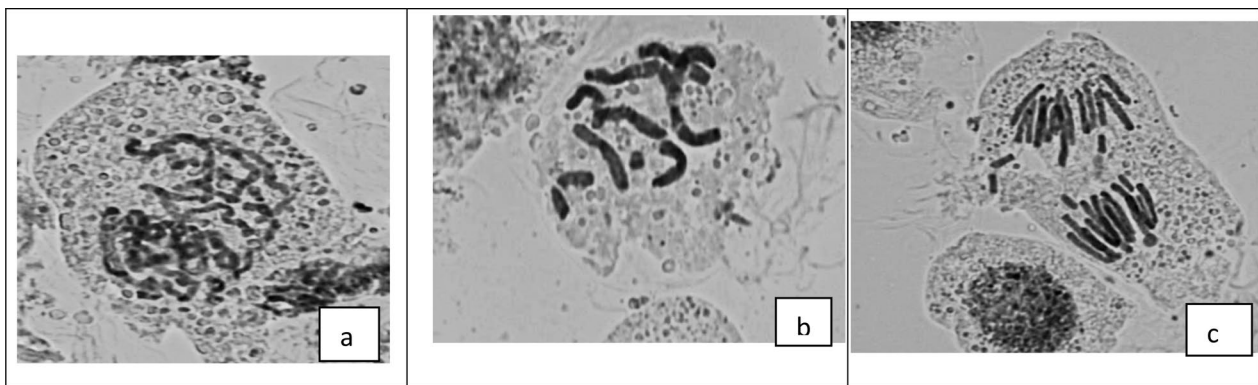


Fig. 6 The effect of 3 h treatment with 25% paracetamol on mitotic chromosomes of *Vicia faba* root tip meristems: irregular prophase (a) disturbed metaphase with fragment and breakage (b), and anaphase with breakage (c)

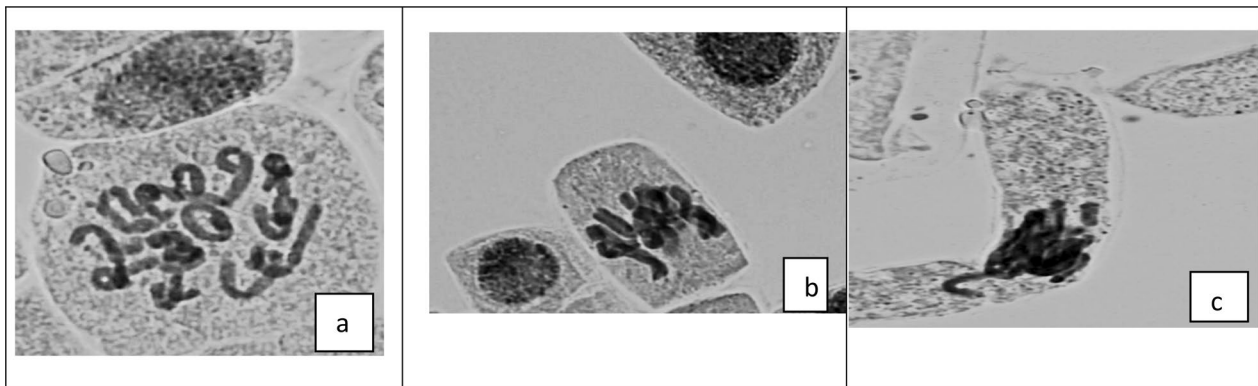


Fig. 7 The effect of 3 h treatment with 50% paracetamol on mitotic chromosomes of *Vicia faba* root tip meristems: c-metaphase with looped ended chromosomes and ring chromosome (a), sticky metaphase with forked end chromosomes (a) and clumped metaphase (c)

The maximum liquefaction percentage was 57.31% scored after the direct treatment with 50% paracetamol, the maximum turbo-genic effect was 71.07% after 12.5% paracetamol after 3 h of direct treatment and it induced maximum in disturbances percentage to reach 66.16%

after 6% paracetamol, and maximum effect on the chromosome liquefaction to reach 59.47 after 50% of paracetamol after 6 h of treatment.

The recovery experiment showed the non-dominant effect of paracetamol treatment on the mitosis mechanism.

3.1 Transmission electronic microscope (TEM) study

The ultra-thin section images captured by transmission electronic microscope; prepared from meristematic root tips of *Vicia faba* root after treatment with varying concentrations and durations of paracetamol revealed interesting changes in cell structure shown in plates 1–4.

Nucleus [N], mitochondria [M], partially degraded mitochondria [dM], dictyosome [D], plastid [Pl], lysosomes [L], peroxisomes [P], endoplasmic reticulum [ER], vacuole [v], autophagic vacuole [AV], gap between the cell wall and cytoplasmic membrane [G] and shrinkage of the cell content away from cell wall were captured after treatment with 6% of paracetamol for three and 6 h [plate 1]. Rising the concentration to 12.5% reflects its sheds on the mitochondria as the partially degraded mitochondria, ring-shaped mitochondria with autophagy new function, fused mitochondria were recorded, while the other cytoplasmic organelles did not show remarkable changes [plate 2].

TEM micrographs showed the effects of 25% of paracetamol on mitochondria, peroxisomes and the cell wall as it showed: partially degraded mitochondria, ring-shaped mitochondria with autophagy new function, fuzzy-shaped mitochondria, unknown ringed organelles with peroxisomes, irregular cell wall in addition to many starch granules [plate 3].

The highest used concentration 50% of paracetamol induced a remarkable shrinkage of the cell content away from cell wall [plate 4]. These findings suggest that paracetamol treatment may have an impact on cell structure and organelle integrity.

4 Discussion

Despite that, the observed increase in the mitotic index after direct treatment with paracetamol in the lower used concentrations came on contradiction with [26] who recorded the concentration-dependent decrease in the mitotic index after treatment with (7.81, 15.62, 31.25, 62.50, 125, 250, 500 and 1000 ppm) and referred this lowering in mitotic index to the direct effect of paracetamol which bind directly to the DNA and the fragmentation effect on the chromosome and the prevention of repairing of damaged DNA, and stated that the unrepaired DNA failed to break the G1 phase in cell cycle.

In our point of view, this contradiction may be due to the high concentration we used which enhanced and stimulated the plant defense mechanism. Our suggestion based on the previous work by [27] found that paracetamol may enhance the plant immunity on two successive

steps. First by production of N-acetyl-p-benzoquinone imine (NAPQI) (a reactive metabolite) that interacts with proteins and nucleic acids, this highly reactive metabolite is conjugated to the nucleophile, reduced glutathione catalyzed by glutathione S-transferases which accelerate glutathione conjugation and thereby usually serve to protect the cell. Second by the detoxification of metabolites that are formed under the conditions of oxidative stress. This second assumption came in great accordance to Husain et al., [13] who recorded an increase in some antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in carrot plant as a consequence to study the pharmaceutical effluent (Fig. 8).

On the other hand, the paracetamol fate within the cell should have its impact in agree with [11] who reported that, 6 h after incubation with 1 mM of acetaminophen (paracetamol), resulted in 18% paracetamol, 64% paracetamol–glucoside, 17% paracetamol glutathione and 1% of the corresponding cysteine conjugate as related metabolites in the root cells of *A. rusticana*. This conjugated cysteine produced in the plant cell systoles plays a significant role in the establishment and signaling of the plant defense compounds such as glucosinolates, thionins or phytoalexins and enhances the plant immune response.

The recorded expansion of prophases and metaphases may reflect the difficulty of the cell passes through to the second check point between the metaphase and anaphases which assure the correct alignment of healthy chromosomes on the equatorial plate to be equally divided between the two daughter cells in a cyclin kinases dependent mechanism [28]. This deduction seems to be very reasonable regarding the induced abnormalities as the paracetamol treatment showed more effectiveness on the spindle formation and chromosomes mobility than it affected the chromatin viscosity on the level of the three lower concentrations (6, 12.5 and 25%). Our deduction is highly agreed with [28, 29] who stated that paracetamol exerts a remarkable effect on the spindle fibers in human V79 cells. [28] referred this spindle alteration to the N-acetyl benzoquinone imine (NAPQI) as active intermediate binds to thiol groups has its potential for binding to tubulin disturbs the spindle function.

Also, the change in the phases' duration as a consequence to the direct treatment may refer to the lower energy level of the dividing cells than required in assumption which is empathized with the TEM investigations and the captured images recorded the partially degraded, ring in shape and swollen mitochondria; the main organelles responsible to supply the cell with energy to carry out the vital process such as division and growth. The mitochondria also are the organelles responsible for ATP production from ADP through electron transport mechanism; this ATP is very essential to the CDKs functioning

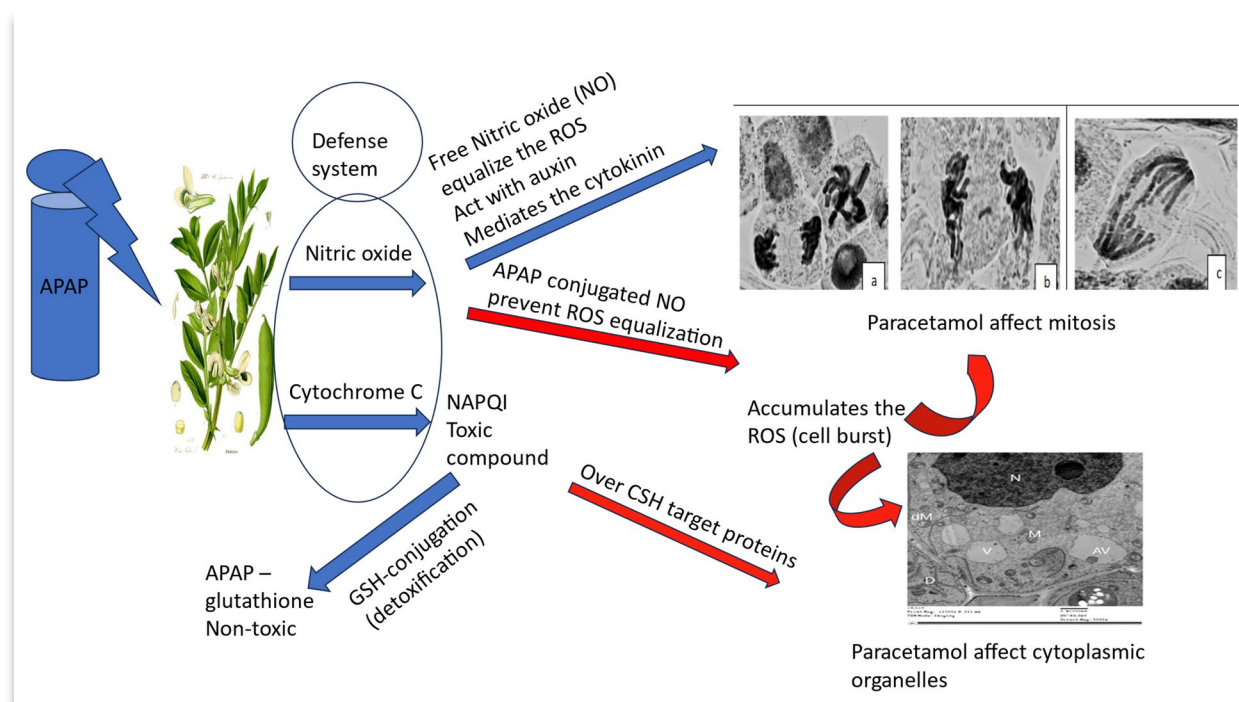


Fig. 8 Summary on the effect of paracetamol remains on the plant mitosis and the cell compartments. (Red arrows represent the harm effect of high concentration over the antioxidant enzymes on the plant). Blue arrows represent the plant defense response through conjugation with antioxidant enzymes)

cell cycle progress by transferring phosphate groups from ATP to specific stretches of amino acids. The other altered organelles induced after direct treatment refers to change in the energy level and the homeostasis of the cells and refers to the plant disable to do its vital process in norm.

It is well known that the normal rising the ROS products within threshold as a result to plant breeze are approved to be one of main regulators of mitosis [29, 30], yet the over-production has its reverse action and drive the cell to self-suicide; on previous study, treatment with paracetamol as pharmaceutical compound was reported to exhibit an oxidative stress and accumulates the ROS over its threshold [13].

Plants have their own antioxidant mechanisms to quench the excess ROS, thereby providing a shield against oxidative stress. One of these defense mechanisms is the NO (nitric oxide) production. (The over-production of ROS as an oxidative burst is normally equalized with the production of NO nitric oxide to keep the normal homeostasis.)

NO is known for its crucial role as significant signaling molecule regulate the plant growth, development and responses to biotic and abiotic stresses and produced in different plant organelles, including chloroplasts, mitochondria, peroxisomes, cytoplasm, cell wall and cell

membrane based on that this work admit the TEM investigations to carry a descriptive analysis to the structure of some of these organelles [30, 31].

On regarding the paracetamol in a study carried by [31, 32] pointed to the interference between acetaminophen and NOS (nitric oxide synthase), this conjugation minimizes the NO production and prevents it from equalizing the ROS over-production and as a consequence allows the devastation of the molecular and cellular components due to the bimolecular oxidation. The limitation in NO production in turn has its impact on the cellular compartments in a process hinder or even alter the production of plant hormones and metabolites that are required to deliver normal growth.

Also, the NO found to have its synergistically action with auxin as growth hormone as activator to the mitosis activation besides it mediates the (CK) cytokinin' functions. Cell cycle also goes smooth from phase to another with the CDK (cyclin-dependent kinase) level's input signal. The level of CDK is fluctuated from very low at G₀ phase, increased to enter the G₁ phase and to little activate B-type cyclin-CDK activity which are sufficient to trigger S phase, and slow down the G₂ phase to prevent the premature mitosis. Full CDK activation triggers mitosis and activates anaphase promoting complex (APC), which triggers anaphase by breaking up the cohesion

complex that holds the two sister chromatids with each other, and also, it feeds back to inactivate CDK allows exit from mitosis and the re-establishment of interphase chromosome and nuclear structure in G_1 phase [32]. Based on these, we can correlate the with the paracetamol treatment with the mitosis system.

The transmission electronic microscope investigation showed that the mostly suffered organelles were the mitochondria; the partially degraded, ring-shaped mitochondria or fuzzy-shaped mitochondria recorded after the higher concentrations of paracetamol gave evidence for its damaging effect and trigger the autophagosome cellular program for cell death; as paracetamol rises the ROS production in the mitochondria over the normal threshold leads to lipid peroxidation and causes deformation in the mitochondrial membrane integrity leading to the release of Cytochrome C from the inter-membrane space to the cytosol [32, 33].

Water contaminated with paracetamol absorbed by seedling's roots is metabolized by the oxidative cytochrome enzyme system into N-acetyl-p-benzoquinone imine (NAPQI), a major toxic metabolite, this toxic metabolite loses by conjugation with GSH (glutathione). The extra paracetamol over available GSH targets cysteine rises the risk of mitochondrial DNA damage and the opening of the mitochondrial permeability transition pore. [33, 34] in mini review on the acetaminophen on liver vote for this damaging effect on mitochondria.

This deduction also can explain a decrease in the mitotic index after 24 h of recovery as a sign on the severe stress of paracetamols which stimulated the plant defense mechanism, and the plants later adjusted normalization to some extent to show the reversible damaging effect. This came in agree with [34, 35] who pointed to the cucumber plant recovery after 48 h and referred his finding to the glutathione S-transferase enzyme activity was significantly increased after the exposure to acetaminophen, while levels of GSH decreased by 55.4% in roots.

Ali et al. [35] referred the deformation in mitochondria structure or integrity to the accumulation of ROS over the non-enzymatic antioxidants (free amino acid and soluble sugar contents) and the enzymatic antioxidants (superoxide dismutase and phenylalanine ammonia-lyase SOD& PAL) as a plant defense against abiotic stress, and pointed to this alteration's impact on the plant cells' energy level which is stimulated the plant to grow.

Dictyosomes are vital in mitosis and cell division in cytokinesis as: a transport system for the synthesized protein to where to do specific functions, a secretion system and formation of lysosomes from the separating cisterna. The observed dictyosomes with swollen cisternae

after treatment may reflect to the paracetamol flow within the cell [36].

Peroxisomes points to the stressful condition of the plant under stress, as the number of peroxisomes in plant reflects its defense condition. Peroxisomes produce ROS such as $O_2^{\cdot-}$ from NADH oxidation and Cyt b localized in peroxisome membrane to be released into the cytosol [37].

The gap between the cell wall and cytoplasmic membrane observed after treatment with the higher concentration 50% and maximum duration 6 h. refers to the paracetamol conjugation with the lipid constituent of the cell wall that alter the cell permeability, and as a consequence this alteration would act as a defense mechanism to preserve the cell wall water potential [26].

Another observation with TEM is the **Starch granules** as a defense response it reflects the cell fear from starvation tends to accumulate and transports of substances through mitochondria pores [38].

In agree with [39] the induced alteration in vital organelles after treatment with the tested material refers to plant response as self-defense in crop plant.

Our findings are documented with the TEM taken images and came in coordination with [40] Who referred the pharmaceutical stress in the in *V. unguiculata* shoots and roots to the oxidative burst in subcellular compartments such as mitochondria through the accumulation of reactive oxygen species (ROS). These ROS compounds disrupt the cell integrity through damaging lipids which produces malondialdehyde (MDA), a reactive mutagen marks the oxidative stress.

5 Conclusion

In this study, the faba plant reveals the cytogenetics, cytological and molecular adaptations to cope under paracetamol. Paracetamol affected *Vicia faba* plant cells in a dose-dependent manner and induced oxidative stress and genotoxicity in *Vicia faba* plant cells. The observed results revealed that high concentrations paracetamol can pose a threat to chromosomes feature and mobility in mitosis apparatus, in addition to the cytoplasmic organelles that affect the energy flow within the plant cells. Therefore, it is important to monitor and regulate the presence of paracetamol and other pharmaceuticals in the environment and to develop effective strategies for their removal or degradation from the recycled water as a previous step before being used in plants irrigation.

Abbreviations

ADP	Adenine di phosphate
APAP	Acetaminophen
APC	Anaphase promoting complex
ATP	Adenine triphosphate
CAT	Catalase

CDKs	Cyclin-dependent kinases
Cyt b	Cytochrome b
Cyt c	Cytochrome c
DNA	Deoxyribonucleic acid
ETC	Electron transport chain
GSH	Glutathione
MDA	Malondialdehyde
NAPQI	N-acetyl-p-benzoquinone imine
NADH	Nicotinamide adenine dinucleotide
NO	Nitric oxide
PAL	Phenylalanine ammonia-lyase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TEM	Transmission electron microscopy

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Author contribution

Conceptualization and methodology were performed by R.A. and A.A.; visualization and formal analysis were performed by A.A., R.A. and M.A.; investigation, resources, data curation and writing—original draft preparation were performed by R.A. and M.A.; writing—review and editing were performed by R.A. All authors have read and agreed to the published version of the manuscript.

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