Cytogenetic effect of *Tribulus terrestris* fruit aqueous extract on Chromosome Aberrations and Mitotic Index in Sorafinib treated albino male rats

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Abstract. The study goal to explore the protective impact of *Tribulus terrestris* (Tt) aqueous extract against Sorafinib (Sor) cytotoxicity in adult male albino rats. Thrity-two rats were divided equally into eight groups and were treated orally as follows: the first control group, the 2nd group received Sorafinib, 3 rd group received *Tribulus terrestris* 300 with Sorafinib and 4th group received *Tribulus terrestris* 600 with Sorafinib group 5th received Sorafinib with *Tribulus terrestris* 300, group 6th received Sorafinib with *Tribulus terrestris* 600, group 7th received *Tribulus terrestris* 300 and group 8th received *Tribulus terrestris* 600. The dose of *Tribulus terrestris* was 300mg/ kg BW, 600mg/ kg BW for 4 weeks, while the dose of Sorafinib was 60 mg/ kg body weight for 3 weeks. Cytogenetic study showed significant decreasing ($P<0.05$) in mitotic index in Sor group and significant increasing ($P<0.05$) in *Tribulus terrestris* groups while chromosomal aberrations showed significantly increase ($P<0.05$) in Sorafinib group in comparison with the control group and significantly decrease ($P<0.05$) in *Tribulus terrestris* groups in comparison with the Sorafinib group. The present study demonstrated that *Tribulus terrestris* possesses potential cytoprotective effects against cytotoxicity caused by Sorafinib.

1 Introduction

Herbal remedies are the oldest form of medicines used in human healthcare system [1]. and demonstrate good compatibility with the human body because of their safety, efficacy, cultural acceptability, and few side effects. Among those, *Tribulus terrestris L.* [2]. Tribulus terrestris (TT) is a plant from the Zygophyllaceae family that is used as a solitary medicinal manager or for example a primary or secondary constituent of several chemical preparations and food additions (health food) in China. The dry fruits of TT, as a outmoded herb medicine (often known as "Ji Li"), have been rummage-sale in China for over thousands of the years to protect the liver, activate blood circulation, improve vision, and relieve itching. Modern research have demonstrated that TT has many pharmacologic aids, including anti-inflammatory, an antioxidant, antibacterial, antiaging, and anti-tumor

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actions [3]. TT has been utilized in clinical therapy, chiefly for the deterrence and treating circulatory disorders, ocular infections, increasing male sexual function and diabetes,The chemical contents of the TT fruit largely comprise saponins, a flavonoids, and alkaloids polysaccharides, amino acids, and vitamins); several research have indicated that saponins and flavonoids in TT primarily participate to its pharmacological activity [4]. Oral, small-molecule, broad-spectrum Sorafinib (SOR) is used to treat several cancers, including kidney, thyroid, and liver cancer. This multikinase inhibitor works in a dose-dependent manner to block many kinases, including as members of the Mitogen-Activated Protein Kinase (MAPK) pathway, Platelet-Derived Growth Factor Receptor (PDGFR) signaling pathway, and Vascular Endothelial Growth Factor Receptor (VEGFR) [5]. Apoptotic effects, angiogenesis, and proliferation are also inhibited by it [6]. Regretfully, rashes, exhaustion, diarrhea, hair loss, nausea, and other severe side effects that lower quality of life, like hand-foot syndrome, are common with SOR [7]. Chromosomal aberration which are generated in chromosome due to alteration in genetic materials through loss, gain or rearrangement of particular segments, External variables such as exposure to sunshine, ionizing radiation, and poisonous substances can produce aberrations. In contrast, internal variables include replication mistakes and uneven cell divisions [8]. A primary source of genetic illnesses, chromosomal aberrations (CA) are abnormalities in the normal number or structure of chromosomes. Monosomy and trisomy are caused by numerical aberrations, which include the loss or gain of a whole chromosome. Chromosome segments are impacted by structural aberrations, which often indicate a chromosomal break and result in distinct rearrangements [9]. Numerous aberrations, including dicentrics, polycentrics, rings, and multiple acentric fragments, are present in some of the injured cells [10]. This study aims to evaluate the cytoprotective effect of aqueous extract of *Tribulus terrestris* against the adverse effect and damage in bone marrow cells induced by sorafenib in rats.

2 Material and Method

2.1 The Plant *Tribulus terrestris L*

The fruit of *Tribulus terrestris* L was purchased from the local market of Hilla city and described and authenticated kindly identified as *Tribulus terrestris* L. by Ministry of Agriculture\ Directorate of seed Testing. For the purpose of the experimental investigation, the fruits were well cleansed and washed to get rid of any dirt or contaminants. They were then air dried at room temperature in the dark and ground into a fine powder.

2.2 Plant Extraction

The hot boiled aqueous extract was prepared by dissolving 100 g of dry plant powder in an erlenmeyer flask and adding 500 ml of boiling distilled water. Then the mixture was shaken for two hours using an electric shaker before leaving it at room temperature for 24 h. The mixture was filtered with four layers of gauze and placed in a tube, then the solution was placed in a centrifuge for 10 min at 2000 rpm. Millipore (0.22 μm) filters were used to filter the supernatant. The filtrate combination was concentrated in the oven for 72 hours to produce a crude extract, which was dry. Until it was used, this extract was stored at 4 °C in a sterile, dark container [11]. The aqueous crude extract was dissolved in sterile distilled water to prepare two doses (300 and 600 mg/ kg body weight) which were administered orally to laboratory rats through gavage tube [12].

Drug (Sorafenib): (Nexavar, 200® mg) got from Bayer Healthcare (Leverkusen, Germany) was used. Pills were crushed in a tissue grinder. The resulting powder was varied

with distilled water and practical via gavage to mice by stomach tube.

Dose: A dose of (60 mg/kg body weight) was given orally every day for 3 weeks [13].

2.3 Laboratory Animals

Healthy adult male wistar rats (*rattus norvegicus*) were the laboratory animals utilized to conduct for the research experiments. They were supplied by the animal house belongs to the Faculty of Science/Kufa College, and they weighed between 165 and 260 grams at the beginning of the studies, and they were 2.5 to 3 months old. They were divided into groups, and each group was kept in a separate plastic cage. They were kept in well- ventilated house conditions(temperature 28-31°C , humidity 50-55% , photoperiod: 12h natural light and 12h dark).

2.4 Experimental design

The rats were housed for two weeks in animal house for adaptation to laboratory condition before being used for experimentation. all the animals were alienated into eight groups of four each

Group 1: four control rats were administrated with distilled water 1ml for 3 weeks (as (negative control).

Group 2: four rats were administrated Sorafinib orally at dose 60 mg/ kg body weight for 3 weeks (as positive control).

Group 3: four rats were administrated *Tribulus terrestris* 300mg/ kg body weight orally for 4 weeks before reciving Sorafinib orally at dose 60 mg/ kg body weight for 3 weeks.

Group 4: four rats were administrated *Tribulus terrestris* 600mg/ kg body weight orally for 4 weeks before reciving Sorafinib orally at dose 60 mg/ kg body weight for 3 weeks.

Group 5: four rats were administrated Sorafinib at dose 60 mg/ kg body weight orally for 3 weeks before reciving *Tribulus terrestris* orally 300mg/ kg body weight for 4 weeks.

Group 6: four rats were administrated Sorafinib at dose 60 mg/ kg body weight orally for 3 weeks before reciving *Tribulus terrestris* orally 600mg/ kg body weight for 4 weeks.

Group 7: four rats were administrated *Tribulus terrestris* 300mg/ kg body weight orally for 4 weeks.

Group 8: four rats were administrated *Tribulus terrestris* 600mg/ kg body weight orally for 4 weeks.

2.5 Preparing Solutions

Hypotonic solution, Potassium chloride 0.075 M (kcl)

In order to prepare the Hypotonic solution, dissolve 0,279 mg of KCl salt in 50 ml of distilled water and used directly at 37Co [14].

Fixation Solution (Carnoy's fixative): fixation solution made by combining three parts (100%) absolute methanol with one part (70%) of glacial acetic acid, which is then stored at 4°C. The solution needs to be made freshly and used no earlier than one hour before [15].

Colchicine Solution: The solution was prepared by dissolving tablet directly from a concentration of 500 mg per ml of Colchicine in 1/2 ml of distilled water, and then injected into the peritoneal cavity about 0.1 solution for each animal [15]

The slides examined under high power and oil immersion.

Mitotic index calculated method: MI is calculated by using the following formula:

$$MI = (D/D+ND) *100 \quad (1)$$

D= number of dividing cells (metaphases+ anaphases+ telophase), ND= number of non-

dividing cells [16].

Slides were examining under in height power and oil immersion to determine the chromosomal aberrations in bone marrow cells.

2.6 Statistical analysis

SPSS version 23 was utilized for statistical studies, and the outcomes are presented as the mean plus or minus the standard error of the mean. Using a one-way analysis of variance, the statistical significance of the differences between the control and treatment groups was established. A statistically important change amid collections was defined as $P<0.05$.

3 Results

Table (1) shows that single dose (60mg/kg) of Sorafinib caused a significant reduction ($p<0.05$) in mitotic index (4.36%) after three weeks in comparison with the negative group (5.08%). On the other hand, the results of this experiment are showed in table (1) treatment with Tribulus caused significant increase ($P<0.05$) in mitotic index in Interaction groups which including: (G3)Tribulus terrestris 300mg/ kg +Sora (6.56%), (G4)Tribulus terrestris 600mg/ kg+ Sora (5.50%), (G5) Sora + Tribulus terrestris 300mg/ kg(6.84%),(G6) Sora + Tribulus terrestris 600mg/ kg (5.88%) In comparison with positive control (4.36%).

Table 1. Mitotic index of experimental rats (mean ± S.D)

Groups	Mitotic index (MI) Mean ± S.D	
G1\Control (negative group)	5.08±0.7	
G2\Sorafinib (positive group)	4.36±0.5	**a**
G3\Tribulus 300 mg/Kg+ Sorafinib 60 mg/Kg	6.56±0.4	**b**
G4\Tribulus 600 mg/Kg + Sorafinib 60 mg/Kg	5.50±0.3	**b**
G5\Sorafinib 60 mg/Kg + Tribulus 300 m g/Kg	6.84±0.4	**b**
G6\Sorafinib 60 mg/Kg + Tribulus 600 mg/Kg	5.88±0.5	**b**
G7\Tribulus 300 mg/Kg	5.76±0.9	**c**
G8\Tribulus 600 mg/Kg	6.26±0.4	**c**
L.S.D (0.05)	0.613	

Letter (a) refers to significant difference comparison with the negative group
Letter (b) refers to significant change contrast with the positive group Letter(c) refers to significant change comparison with the negative group
S.D standard.deviation

In addition, the results of chromosomal aberrations experiment indicated that Sorafinib treatment for three weeks induced certain types of structural aberrations which were ring chromosomes, sticky Chromosome, fragmentation chromosome (F), duplication chromosome (DUP), attenuated centromere (Cen.atten) and other types of aberrations included dicentric chromosome, deletion (Del), inversion, translocation, , attenuated chromosome (Chrom.atte), chromatid gap (Chro.gap) , End to End chromosome (E.E), Rose chromosome, table (2) , Figure (A,B,C,D,E,F,K,L) and Also, Sorafinib treatment caused numerical aberration in the form of polyploidy (J). These induced aberrations were statistically significant ($P< 0.05$). While, the aqueous crude extract of *Tribulus terrestris* fruit had the ability to reduce significantly ($P<0.05$) the chromosomal aberrations per 100

cells as shown in table (2). Figure 1 (G, H).

Table 2. Percentage of some chromosomal aberrations of bone marrow cells of experimental rats (Mean±S.D)

Groups	Chr. gap	Ch.br	Cen. atten	Del	DUP	E.E	Ring	F	C.F	Poly pliod y	Stieness	Ch.at ten	Anab rige	Rose	Inversion
G1	Non	Non	10.0± 1.1	1.25± 0.2	0.5± 0.04	0.75± 0.1	Non	8± 1.7	0.25± 0.01	7.0± 1.3	1.5± 0.04	15.75± 2	Non	0.5±0.04	Non
G2	2±0.3	4.5± 0.4 a	16.0± 1.3 a	3.0± 0.8 a	2.0± 0.03 a	1.5± 0.04 a	1.25± 0.2 a	18.0± 4.2 a	1.5± 0.04 a	12.75± 1.9 a	6.25± 1.3 a	34.25± 6.1 a	1.5± 0.04 a	0.25±0.01 a	1.75± 0.6 a
G3	Non	Non b	11.0± 2.1 b	0.5± 0.1 b	1.0± 1.01 b	0.5± 0.04 b	0±0 b	2.25± 0.6 b	0.75± 0.1 b	4.5± 0.6 b	2.75± 0.4 b	13.75± 1.9 b	0.5± 0.04 b	Non b	0.25± 0.01 b
G4	Non	1± 0.2 b	8.5± 1.1 b	1.25± 0.2 b	0.75± 0.1 b	1.25± 0.2 b	0±0 b	1.25± 0.2 b	1±0.2 b	7.5± 1.1 b	Non b	21.75± 3.6 b	Non b	Non b	Non b
G5	Non	Non b	11.5± 1.4 b	0.25± 0.01 b	1.25± 0.2 b	1.0± 0.2 b	0.25± 0.01 b	1± 0.2 b	0.5± 0.02 b	0.0± 0.0 b	Non b	15.5± 3.2 b	0.25± 0.01 b	0.0±0.0 b	0.5± 0.1 b
G6	Non	1.25± 0.2 b	4.0± 0.2 b	1.25± 0.2 b	0.5± 0.04 b	0.5± 0.04 b	0.25± 0.01 b	0.25± 0.01 b	1.25± 0.2	6.0± 0.8 b	Non b	13.5± 2.1 b	0.25± 0.01 b	0.25±0.01	0.25± 0.01 b
G7	Non	0.25± 0.01	9.0± 1.2	0.75± 0.1c	1.5± 0.04 c	1.0± 0.2c	0.25± 0.01c	0.75± 0.1 c	0.75± 0.1	6.25± 0.9	Non c	2.75± 0.4 c	0.25± 0.01	0.25±0.01c	0.5± 0.1c
G8	Non	0.25± 0.01	3.75± 0.5 c	0.5± 0.1 c	1± 0.2 c	0.25± 0.01c	0.75± 0.1 c	3.5± 0.9 c	0.25± 0.01	1.5± 0.04	Non c	4.0± 0.2 c	0.75± 0.1 c	Non c	Non
L.S. D	N.S	0.303	3.399	0.206	0.422	0.182	0.159	0.744	0.319	2.113	0.922	2.488	0.436	0.107	0.206

Letter (a) refers to significant difference comparison with the negative group
Letter (b) refers to significant difference comparison with the positive group
Letter (c) refers to significant difference comparison with the negative group
S.D: standard deviation

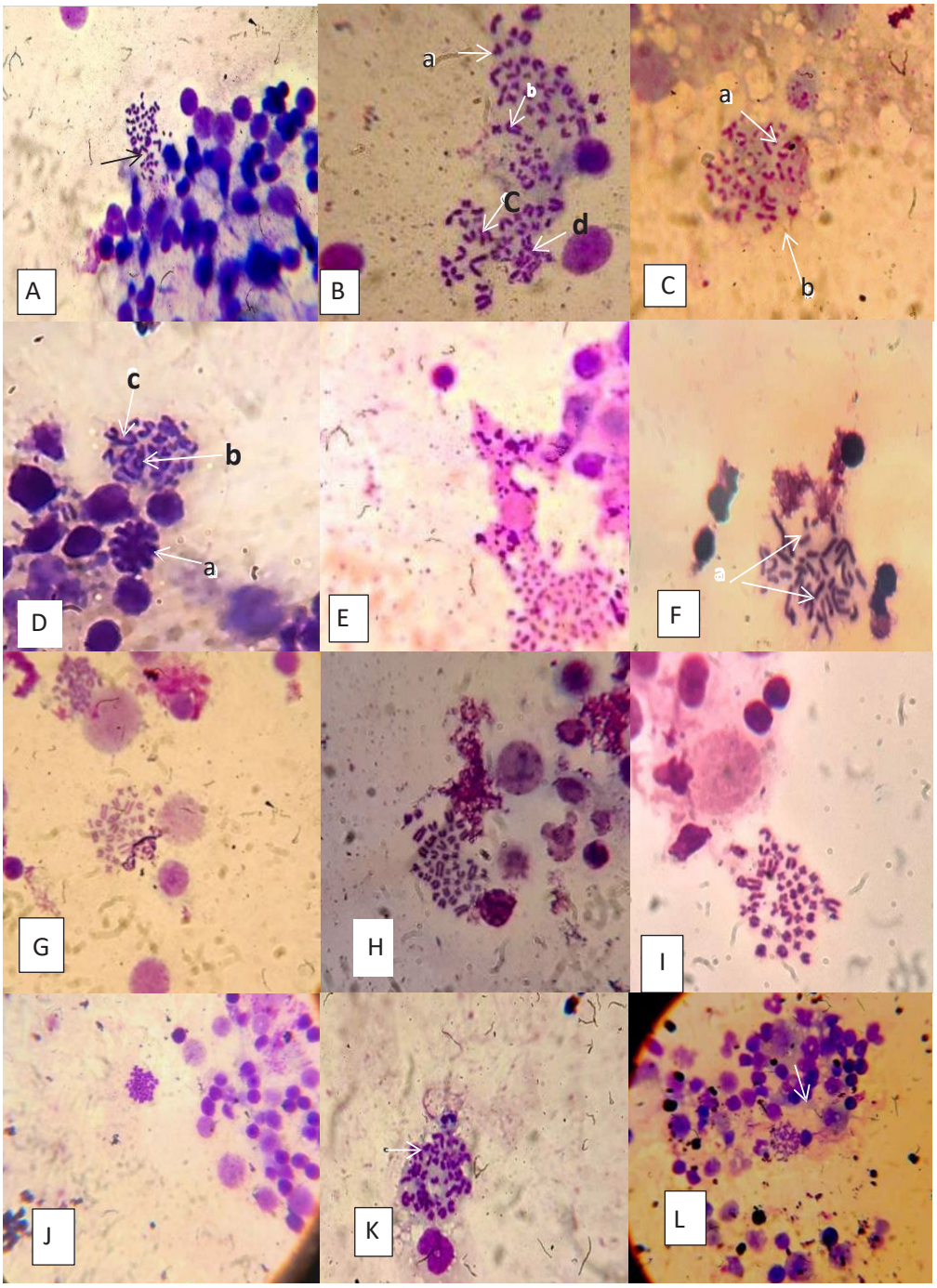

Fig.1. Metaphase chromosomes of bone marrow cells treated rats with Sorafinib.
A-Translocation. B-(a-Fragments chromosome,b-Anaphase bridge, c-Dicentric chromosome, d-inverted insertion). C-(a-attenuted chromosome,b-attenuated centromere).D-(a-Rose chromosome,Ring chromosome,c-End to End chromosome).E-(Fragmentation). F-(Elongation). J-(Polypiody). K-(Delation).L-(Dicentric chromosome).G,H(Metaphase chromosomes of bone marrow cells treated rats with Tribulus terrestris)I-(Control group).

4 Discussion

The Mitotic index is a valuable tool for characterizing cell proliferation and identifying substances that have the ability to inhibit or induce mitotic progression. As such, it is useful for determining the kinetics of cell proliferation in animal models [17]. This technique makes it possible to determine how different chemical and physical factors affect the mitotic response. Additionally, exposure to many genotoxic substances might have a good or negative impact on the mitotic response. These factors include radiation, medication, and herbal remedies [18]. One possible explanation for the Sorafinib group's inhibition of the mitotic index is that DNA synthesis was inhibited. [19], arrest of one or more mitotic phases [18]. The accumulative effect of Sorafinib which increases the cytotoxic effect on bone marrow cells, and this was reflected on the MI. In a study by the researcher [20] demonstrated that sorafenib inhibited the growth of human APL (acute promyelocytic leukemia) cells and induced apoptosis by upregulating the proapoptotic proteins caspase-3 and caspase-8 and downregulating the antiapoptotic protein MCL1 and the essential protein cyclin D1, which is required for the cell cycle to progression.

Studies and scientific research have confirmed that there are many plants that have medicinal effectiveness in reducing the rate of genetic mutations resulting from mutagens and carcinogens. They also work to increase the effectiveness of the immune system, it is believed that Mitosis is increased by substances that stimulate cell division [21] observed that the steroidal saponins in Tribulus exhibited both activation of non-specific immunity in an animal model and immunostimulating action in in vitro studies. Furthermore, Tt demonstrated elevated B cell activity with notable rises in serum antibody titers, which may act as effectors of the humoral response [22]. Or the reason may be the effect of aqueous extracts, which leads to an increase in the amount of nuclear proteins and stimulating the enzyme DNA polymerase responsible for the replication of DNA, which affects cell division or the reason may be that it contains compounds that have the ability to increase the level of antioxidant enzymes [23]. Numerous plant extracts have demonstrated their antitumor effects against a variety of human cancer cells in both in vitro and in vivo experiments due to their high steroidal saponin content [24]. In a recent study, [25] also revealed the cytotoxic effects of methanolic and saponin extracts from leaves and seeds of T. terrestris against breast cancer cell lines (MCF7) and their results suggested that inhibitory activities were contributed to intrinsic and extrinsic apoptotic pathways. Giving the extract before the drug played an important role in reducing the toxic effect of sorafenib. This may be due to the ability of the active compounds in the extracts to change the permeability of the cell membrane of bone marrow cells and prevent the mutagen from reaching the cells. This activity has been demonstrated by compounds that have direct inhibitory activity. The mutagen works to raise the mitotic index of bone marrow cells to the level of the normal state [26]. The extracts bind with the mutagen or with its active metabolites covalently, forming compounds that prevent its entry into the cell [27]. And on this basis, Tribulus are classified as compounds that act inside the cell as antimutagenic When the extract is given after the mutagen, it causes an increase in the value of the mitotic index. The reason for this can be attributed to the extract’s ability to inhibit the inhibitory effect of the mutagen and repair what was destroyed by the mutagen by increasing the efficiency of DNA replication and removing the toxic effects of the mutagen by stimulating the cell’s repair systems [28]. On the other hand,the results of chromosomal aberrations of bone marrow cells experiment indicated that Sorafinib treatment induced certain types of chromosomal aberrations,

Various investigations have indicated that the formation of reactive oxygen species (ROS) could be a potential mechanism underlying this toxicity [29]. Many studies have shown that Sorafenib interaction with subunits of the mitochondrial respiratory system

generates ROS that may promote mitochondrial damage [30]. Generally, the harmful effects of ROS in the cell include : damage on DNA or RNA, lipid peroxidation of polyunsaturated fatty acids (such as membrane phospholipids) and oxidation of proteins [31]. Significant oxidative reactions occur when ROS interacts with deoxyribose and nitrogenous bases in DNA. Mutations, apoptosis, necrosis, carcinogenesis, and inherited illnesses can result from this. Nucleosomes, which are essential for the organization of DNA within chromosomes, rupture, leading to DNA fragmentation. This causes issues with the compaction and coiling of DNA within chromatin. The hydroxyl radical directly damages DNA by oxidizing purine and pyrimidine bases, and chromatin is crucial for controlling gene transcription [32].

While, the aqueous crude extract of Tribulus terrestris fruit had the ability to reduce the chromosomal aberrations, Tribulus terrestris fruit is rich in chemicals, including glycosides, alkaloids, flavonoids, saponins, and sapogenins. These compounds have a variety of therapeutic uses, such as analgesic, antibacterial, anticancer, metal chelating, anti-inflammatory properties and antioxidant [33, 34] found that the phytomolecules found in the saponin-rich butanol fraction of *Tribulus terrestris* fruits have the capacity to counteract the oxidative damage caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity by increasing the levels of both enzymatic and non-enzymatic antioxidants, such as glutathione, vitamin C, and E, as well as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione STransferase (GST). Numerous studies have shown that these polyphenolic compounds have a wide range of activities and the antioxidant capacity is among them [35]. These results in this study were in agreement with the results of [36] which indicated that the oral administration of *Tribulus terrestris* root extract (800 mg/kg body weight) significant reduce in chromosomal aberrations and micronucleus frequencies in mouse bone marrow cells.

5 Conclusion

It can be concluded that the aqueous extract of Tribulus fruits has a high inhibitory activity against the drug sorafenib, as this extract reduces the toxicity of the drug and the rate of chromosomal abnormalities in both types of treatments (before or after), so Tribulus fruits extract can be classified as Desmutagene substances and Bioantimutagene substances, this is consistent with [37] where he pointed out that plant extracts are anti- mutagenic agents outside and inside the cell.

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