Protective Effect of Cape gooseberry Fruit against Mutagenicity of Potassium Bromate in Mice

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Abstract: Cape gooseberry is a widely used medicinal herb for treating cancer, asthma, hepatitis, dermatitis and rheumatism. In the present study, Cape gooseberry fruit juice demonstrated antioxidant activity against mutation induced by potassium bromate. The aim of the present study was to investigate the possible modulatory anticlastogenic effects of pre treatment with 500 mg/kg b.wt and 1 g/kg Cape gooseberry (Cg) fruit juice and post treatment interferon-α (INF-α) and 1g/kg Cg & INF-α on somatic cells (bone marrow cells) of potassium bromate treated mice. 90 Male Swiss albino mice weighing 30-35g, were divided into six groups of 15 animals as follows: control group, 1.6 g/liter (196 mg/kg) KBrO3 group for 15 weeks, two groups of pre treatment with 500mg/kg and 1g/kg Cape gooseberry for 2 weeks before KBrO3 and continuous with it, post treatment with 6.5 ×107/kg interferon-α alone and interferon-α & cape gooseberry for 6 weeks. Mice were received KBrO3 and Cg orally. They were injected with interferon-α subcutaneously. Animals were sacrificed to assay chromosomal aberrations of treated animals. It is noticed that KBrO3 increased the percentage of chromosomal aberrations (very highly significant \(P<0.001\) compared with the control group). The most important result obtained in the present study that this percentage was decreased when Cg, interferon-α and interferon-α & Cg were given before and after KBrO3. The reduction was very highly significant \(P<0.001\) compared with the control group. Our results supposed that the very important components of Cg juice might be responsible for antioxidant activity and prevention of peroxidative damage to protein and DNA which produced chromosomal aberrations. From these findings it is to be suggested that Cape gooseberry as new source of bioactive phytochemicals and functional foods, should be advised before and during chemotherapy of malignant tumors.

Keywords: Potassium bromate; Cape gooseberry; interferon-α

1. Introduction

Potassium bromate (KBrO3) is a by-product from ozonation of high-bromide surface water for production of drinking water [1]. Although KBrO3 is widely contained in food products including production of fish paste, fermented beverages, food additives in the bread-making process for the maturation of flour (because of its oxidizing properties), as a neutralizer in cold-wave hair lotions, due to its carcinogenic potentials, such uses in foods are now prohibited, reducing exposure to humans [2]. Indeed exposures to KBrO3 can cause renal cell tumors in rats, hamsters and mice, and thyroid and testicular mesothelial tumors in rats [3, 4] as well as causing DNA strand breaks and poly (ADP) ribosylation in the kidney that is associated with proliferative responses [5]. The toxicological effects may be mediated via the induction of oxidative stress [6-8].

The use of chemotherapy in the treatment of cancer has opened new possibilities for improvement of the quality of life of cancer patients and even for the cure of disease. Despite its successes, treatment with many anti cancer drugs have been shown to be mutagenic, teratogenic, carcinogenic and associated with second malignancies. Interferons (IFNs) are an important treatment for a number of solid tumors and hematological malignancies. These include melanoma, renal cell carcinoma, AIDS, follicular lymphoma, hairy cell leukemia. In addition, IFN therapy is associated with significant side effects which have an impact on the patient’s quality of life and the physician’s ability to optimally treat the patient [9].

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, or parasites or tumor cells. Moreover, they allow communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells [10]. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells and they increase the ability of uninfected host cells to resist new infection by virus.

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Interferon α-2a is one of the type I of interferon that bind to a specific cell receptors [10, 11].

One of the approaches to deal with this problem is to search for suitable anti-mutagens. Over the last few years, much attention has been paid to the research of naturally occurring agents that are able to stimulate defense mechanisms of the organism. Among these agents is The Cape gooseberry (Physalis peruviana L.) which belongs to the class of drugs known as biological response modifiers. The Cape gooseberry is the second highest fresh fruit export in Colomba. Because of its nutritional and medicinal attributes, it is attractive for international markets. Colomba is the top producer of Cape gooseberry worldwide followed by South Africa. In addition, the origin of Cape gooseberry is the Andean highland of Northern South America. Moreover, Cape gooseberry has been grown in Egypt, New Zealand, Australia and Great Britain [12, 13]. The fruits, the part of the plant that is commercialized, are contained in a calyx and are characterized for their nutritional value [14]. They are gaining renewed interest as an ingredient for production of functional foods due to their concentration of bioactive compounds, such as provitamin A, vitamin C, iron, ascorbic acid and some of the vitamin B-complex. Moreover, there are many classes of phenolic compounds. The protein and phosphorus levels in the fruits are exceptional as well as pectin that is used in jum production [13]. The fruit contain 15% soluble solids (mainly sugars) and its high level of fructose. Its high content of phosphorus level and dietary fiber, wherein fruit pectin acts as an intestinal regulator [12, 13].

The aim of the present work is to study the protective effects of Cape gooseberry and interferon-α against genotoxic and cytotoxic effects of potassium bromate under in vivo conditions in mice.

2. Materials and Methods

Animals

Male Swiss albino mice (Mus musculus) aged 9-11 weeks and weighing 30-35g was used in all experiments. Mice were housed in plastic cages with stainless steel grill tops, bedded with rice husk. 90 mice were divided into six experimental groups of 15 animals in different cages. They were maintained under proper environmental conditions. Mice were provided with standard diet and tap water. They were acclimatized to laboratory conditions for at least 7 days before the experiment.

Experimental doses

Cape gooseberry (Physalis peruviana)

Cape gooseberry juice yield is 72.6% of the berry weight. In gooseberry, quercetin is the main phenolic compound, followed by myricetin and kaempferol [15]. Good amounts of phenolics were estimated in Cape gooseberry juice. Ascorbic acid level in Cape gooseberry (46 mg/100 g). Fruit juice was found to contain 0.2% oil, wherein linoleic acid, oleic acid, palmitic acid, γ-linolenic (GLA) acid and palmitoleic acid were the main fatty acids. Vitamin E level was high, wherein γ- and α-tocopherols were the main constituents. High amounts of β-carotene were detected also in the juice.

Selection of Cape gooseberry doses

Cape gooseberry (Cg) was administered to mice by gavage, at doses 500 mg and 1 g/kg daily [16] for 2 weeks (14 days) [17].

Preparation of the doses for treatment

Cape gooseberry was purchased from Egyptian local markets. Fruits were first washed thoroughly to remove impurities. After washing the fruits were cut into small pieces. Pieces placed in the blender to make a juice (500g Cg juice up to 500ml dist. water) where each 1 ml juice contain 1g Cg. The stock was distributed into small tubes and preserved in the refrigerator for the time of use. The juice was shaken well just before oral administration by oral tube.

Potassium bromate

Potassium bromate (KBrO₃) is a white crystalline powder, which is colourless, odourless, and tasteless with a molecular mass of 167 g. Potassium bromate is widely used as a food additive in the bread-making process for the maturation of flour and as a neutralizer in cold-wave hair lotions. It is also found in drinking-water samples as a by-product of ozone disinfection. It is prepared chemically as 2KBr + 3O₂→2KBrO₃ [18].

Selection of doses of potassium bromate

The recommended dose was 1.6 g/liter (196 mg/kg b.wt) daily [3] for 15 weeks [19, 20]. The rationale for selection of this dosage is based on the LD₅₀= 280–355 mg/kg of body weight for bromate intoxication reported in mice [3].

Preparation of doses for treatment

Potassium bromate present in the form of white crystal powder. The bottle was contained 500 g and was purchased from EL-Gamhuria Company, Egypt. We used 1.6 g and were dissolved in liter of distilled water. Mixture was preserved in brown glass water bottles [8]. Mice received the potassium bromate orally by oral tube.

Reiferon (interferon α-2a)

Interferon is used extensively as an antiviral or antineoplastic agent. Interferon (a human leukocyte protein moiety reduced). A type I interferon consisting of 165 amino acid residues with lysine in position 23 (C₅₆₀, H₁₅₃, N₂₂₇, O₂₅₅, S₈). This protein is produced by recombinant DNA technology and resembles interferon secreted by leukocytes [21].
Reiferon (interferon α-2a) was purchased from EL-Esaph pharmacy, Egypt. The box was contained 6 ampoules, each one contain 3 M.IU/1ml. It was administered to mice subcutaneous injection at dose $6.5 \times 10^5$/kg three times weekly for 6 weeks (42 days) [22].

**Chromosomal analysis**

**Chemicals**
Colchicine 0.04% solution (Adwics).
Phosphate buffer saline (PBS).
Hypotonic solution: 5.6 gm KCL dissolved in 1liter dist. H$_2$O (0.56%).

**Fixative:**
Glacial acetic acid: absolute methyl alcohol 1: 3 was freshly prepared at the time of experiment.

**Stock solution of Giemsa stain:**
1gm Giemsa powder (Adwic Co.) dissolved in 50 ml glycerine, the mixture was incubated at 60°C for 2 hours. After cooling, 50 ml of absolute methyl alcohol was added. The mixture was kept in a clean bottle and filtered before use.

**Preparation of metaphase chromosomes**
Metaphase chromosomes were prepared from bone marrow cells of control and treated animals [23].

**Staining**
The slides were stained in 5% Giemsa for 10 minutes.

**Analysis of the slides**
For each mouse, 50 metaphases were analyzed for chromosomal aberration in light research microscope with 1000 x magnification [24].

**Statistical analysis**
Incidence of abnormal metaphases was analyzed for significance by student, $t$-test [25, 26].

3. Results
Mice were received potassium bromate (KBrO$_3$) orally for 15 weeks and treated with chemotherapy (interferon-α) and natural therapy by Cape gooseberry.

Potassium bromate induced high level of chromosomal aberrations ($P<0.001$) as compared with control group 7%. The frequency of chromosomal aberrations (CAs) 92.67% (Table 3 and Fig. 3).

The major types of SCAs detected in our experiments 76% were deletion, centric fusion, centric separation, chromatid exchange were predominantly (Fig. 4). Diradial, pulverization, isochromatid gap and dicentric were became next. Whereas endoreduplication, break and ring were hardly speculated (Fig. 5). These results graphically represented in Fig. 1 and Table 1.

In addition, numerical changes increased 16.6% as compared with control group 3%. Hypodiploidy was significantly increased as compared with untreated group (where the frequencies of hypodiploidy were more than hyperdiploidy) Fig. 6. Hyperdiploidy increased but non significant (Table 2) and graphically represented in Fig. 2.

First : Treatment with interferon-α alone, interferon-α and 1g/kg cape gooseberry together for 6 weeks after exposure to KBrO$_3$ for 15 weeks decreased the percentage of chromosomal damages to 65% and 54.33% respectively as compared with KBrO$_3$ group 92.67%.

The present study demonstrated that the frequency of chromosomal damages reduced in treatment with interferon-α and 1g/kg Cape gooseberry together more than treatment with interferon-α alone.

Second: Treatment with 500mg and 1g/kg Cape gooseberry (Cg) for 2 weeks before exposure to KBrO$_3$ and continuous with KBrO$_3$ for 15 weeks decreased the percentage of CAs to 75.67% and 55% respectively.

Since our results showed that the protective effects take place in high dose (1g/kg) more than low dose (500mg/kg) of Cg. Frequency of total structural and numerical chromosomal aberrations were reduced by increasing the dose of Cg. This assured the dose dependent relationship. On the other hand, treatment with low or high dose of Cg increased the health of mice more than other received KBrO$_3$ alone.

4. Discussion
In the last few years, there has been growing interest in substances that can protect or minimize the damage to genetic material caused by various contaminants to which humans are exposed. Therefore, the present study investigated the antigenotoxic effects of Cape gooseberry alone or Cape gooseberry and interferon-α. The antigenotoxic effects were determined against the DNA damage induced by potassium bromate to which patient are exposed. Furthermore, different treatment protocols were used to determine the possible mechanism of antigenotoxicity through which Cape gooseberry could prevent DNA damage.

The fruit has been used as a good source of bioactive constituents, such as provitamin A, minerals, vitamin C, vitamin K and vitamin B-complex. In gooseberry, quercetin is the main phenolic compound, followed by myricetin and kaempferol [15]. High amounts of β-carotene and withanolides were detected in the juice.
Table 1: The percentage of structural chromosomal aberration in inducted mice with 195 mg/kg potassium bromate for 15 weeks and treated with interferon-α and Cape gooseberry.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Structural chromosomal aberration (SCA)/300 metaphase</th>
<th>Total SCA</th>
<th>M ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KBrO₃</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500mg Cg + KBrO₃</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1gm Cg + KBrO₃</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>KBrO₃ + I</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
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<tr>
<td>KBrO₃ + Cg</td>
<td>Br. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
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</table>

C.S. = Centric separation; C.F. = Centric fusion; Del. = Deletion; Dicen = Dicentric; Br. = Break; Iso-g = Isochromatid gap; Dir = Diradial; Chrex = Chromatid exchange; Pulv. = Pulverization; Endo. = Endoreduplication; SD = Standard deviation; SE = Standard error; M. = Mean; Cg = Cape gooseberry; I = Interferon-α

Table 2: The percentage of numerical chromosomal aberration in inducted mice with 195 mg/kg potassium bromate for 15 weeks and treated with interferon-α and Cape gooseberry.

<table>
<thead>
<tr>
<th>Groups</th>
<th>NCA</th>
<th>Total NCA</th>
<th>M ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KBrO₃</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500mg Cg + KBrO₃</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1gm Cg + KBrO₃</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KBrO₃ + I</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>KBrO₃ + Cg</td>
<td>Hypo. 0 0 1 0 2 3 3 0 0 1 0 0 0 0 12 2 0.63 0.26 4%</td>
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</table>

NCA = Numerical chromosomal aberrations

Table 3: The percentage of structural and numerical chromosomal aberration in inducted mice with 195 mg/kg potassium bromate for 15 weeks and treated with interferon-α and Cape gooseberry.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total SCA %</th>
<th>Total NCA %</th>
<th>Total Aberr.</th>
<th>M ± SD</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12 4%</td>
<td>9 3%</td>
<td>21</td>
<td>3.5±0.547 0.22 7%</td>
<td></td>
</tr>
<tr>
<td>KBrO₃</td>
<td>228 76%</td>
<td>50 16.6%</td>
<td>278</td>
<td>46.3±1.03 0.422 92.67% ***</td>
<td></td>
</tr>
<tr>
<td>500mg Cg + KBrO₃</td>
<td>193 64.3%</td>
<td>34 11.3%</td>
<td>227</td>
<td>37.83±1.7 0.70 75.67% ***</td>
<td></td>
</tr>
<tr>
<td>1gm Cg + KBrO₃</td>
<td>134 44.7%</td>
<td>31 10.3%</td>
<td>165</td>
<td>27.5±2.17 0.88 55% ***</td>
<td></td>
</tr>
<tr>
<td>KBrO₃ + I</td>
<td>125 41.7%</td>
<td>70 23.3%</td>
<td>195</td>
<td>32.5±1.64 0.67 65% ***</td>
<td></td>
</tr>
<tr>
<td>KBrO₃ + Cg</td>
<td>112 37.3%</td>
<td>51 17%</td>
<td>163</td>
<td>27.2±2.7 1.14 54.33% ***</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 The percentage of structure chromosomal aberrations in induced mice with potassium bromate (KBrO₃) and treated with Cape gooseberry (Cg), interferon-α (I) and interferon-α & Cape gooseberry.

Fig. 2: The percentage of numerical chromosomal aberrations in induced mice with potassium bromate and treated with Cape gooseberry (Cg), interferon-α (I) and interferon-α & Cape gooseberry.

Fig. 3 The percentage of chromosomal aberrations in induced mice with 195 mg/kg potassium bromate for 15 weeks and treated with Cape gooseberry (Cg), interferon-α (I) and interferon-α & Cape gooseberry.

Fig. 4 Show normal metaphase (a) and various types of structural chromosomal aberrations were induced by KBrO₃ where D (deletion), Chrex (chromatid exchange), CS (centric separation), CF (centric fusion) and Dir (diradial)

Fig. 5 Demonstrated various types of structural chromosomal aberrations were induced by KBrO₃ where Dir (diradial), b & c (partial and complete pulverization), IG (isochromatid gap), Dic (dicentric), End (endoreduplication), B (break) and R (ring).
In the present investigation, mice treated with 195 mg/kg potassium bromate for 15 weeks presented a very highly significant increase in total number of chromosome aberrations (CAs) when compared to the control group 7%. This suggests that potassium bromate metabolism involves increased lipid peroxidation and the generation of intermediates and bromine oxide-radicals that attack DNA; oxidative damage to sugars or bases in DNA can ultimately result in double-strand breakage. Intermediates (metabolites) that can react with DNA resulting in single, double-strand breakage and chromosomal damage.

Therefore, KBrO₃ induced the high rates of chromosomal aberrations. Also, previous studies showed that bromate can damage DNA both in vitro and in vivo [1, 3, 6-8, 27]. It can induce both point mutations and chromosomal mutations, but it is a weak point mutagen and is a potent chromosomal mutagen. All of the data suggest that bromate may be a genotoxic carcinogen, inducing mutations and other genetic damage through loss of heterozygosity (LOH) resulting from DNA strand breaks and deletions induced by oxidative DNA damage as the predominant primary lesion. Data in our study revealed that, the major types of CAs detected in our experiments were deletion, centric fusion, centric separation, chromatid exchange were predominantly. Diradial, pulverization, isochromatid gap and dicentric were became next. Whereas endoreduplication, break and ring were hardly speculated. It may be due to the metabolites of KBrO₃. On other hand, KBrO₃ can induce chromosomal aberrations in cultured cells, Chinese hamster lung (CHL) cells treated with 0.0625–0.25 mg/ml KBrO₃ without metabolic activation had significantly higher rates of chromosome aberrations than the controls [28]. In addition, Chinese hamster DON-6 cells treated with KBrO₃ showed a positive response for aberration induction [29]. The main aberration types observed were chromatid type breaks and chromatid exchanges. The clastogenic activity of KBrO₃ was considered to be relatively strong [30, 31]. A similar result also was found in V79 cells exposed to KBrO₃.

In the present study, low or high dose of Cape gooseberry decreased the frequencies of chromosomal aberrations. While high dose was more effective than low dose. This suggests that Cape gooseberry induced a dose dependant. This result in agreement with previous investigators demonstrated that Physalis peruviana (Cg) fruit juice exhibited a dose dependent reduced growth rate and cellular proliferation [17]. The protective effect of Cape gooseberry may be the possible involvement of its antioxidant and scavenging properties. This suggests that phenolic compounds, withanolides and other components in Cape gooseberry have antioxidant activities and their ability to scavenge free radicals, break radical chain reaction and chelate metals. This result in coincided with others reported that P. peruviana contain high amount of withanolides and phenolics which have a strong antioxidant property and prevent peroxidative damage to liver microsomes and hepatocytes [32]. In addition, Withanolides exhibited a broad spectrum of biological properties and significant pharmacological activities, including insect-antifeedant, insect-repellent activities, hepatoprotective, immuno-modulatory, antibacterial, anti-inflammatory, antitumor, cytotoxic activity, and protection against CCl₄-induced hepatotoxicity [32-34]. Also, various flavonoids such as quercetin could protect DNA both by reducing oxidative DNA damage and by enhancing DNA repair through modulation of DNA repair enzymes expression [35]. Moreover, ascorbic acid to inhibit reactive oxygen species production, but not alter Bro₃⁻ (bromate)-induced nuclear fragmentation or 8-hydroxydeoxyguanosine (8-OHdG) formation [27], is somewhat in contrast to studies demonstrating that ascorbic acid reduces bromate induced formation of 8-OHdG in vivo [36]. Moreover, extract of physalis exerted anticancer effect due to a combination of apoptotic and autophagic cell death mechanisms on Caov-3 (human ovarian carcinoma) cells [37, 38].

Our study demonstrated that the frequency of chromosomal damages reduced in post-treatment with interferon-α. This suggested that interferon-α
may stimulate mechanism defense of immune system which kills infected cells by promoting apoptosis. These results in accordance with previous investigators reported that the infected cells can warn neighboring cells by releasing interferon so neighboring cells produce large amount of enzyme (protein kinase) [39, 40]. These enzymes inhibit protein (eIF-2) synthesis and destroy both viral and infected host cells. In addition, interferons also limit viral spread by increasing (p53) protein activity, which kills virus-infected cells by promoting apoptosis [41]. The effect of IFN on p53 is also linked to its protective role against certain cancers.

The present study demonstrated that the frequency of chromosomal damages reduced in treatment with interferon-α and 1g/kg Cape gooseberry together more than treatment with interferon-α alone. This suggested that post treatment with interferon-α and 1g/kg cape gooseberry might increase the intracellular content of antioxidant compounds, thus intensifying the protection against damage induced by free radicals, reactive oxygen species (ROS), which may react with protein and DNA producing chromosomal aberrations.

Conclusion
Finally, it can be concluded that potassium bromate induced the high rates of chromosomal aberrations. The major types of CAs detected in our experiments were deletion, centric fusion, centric separation, chromatid exchange were predominantly. Diradial, pulverization, isochromatid gap and dicentric were became next. Whereas endoreduplication, break and ring were hardly speculated. It is important to note that pre treatment with low or high dose of Cape gooseberry decreased the frequencies of chromosomal aberrations (Cape gooseberry induced a dose dependant). Moreover, post treatment with interferon-α and 1g/kg Cape gooseberry together reduced frequency of chromosomal damages more than post treatment with interferon-α alone.

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